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THE USE OF ARTIFICIAL TRANSMURAL POTENTIALS
IN VASCULAR PROSTHESES TO PREVENT THROMBOSIS

by



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A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "The Use of Artificial Transmural Potentials in Vascular Prostheses to Prevent Thrombosis" submitted by Donald Bruce Quinn in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

This thesis deals with the use of a synthetic material for prosthetics to be used in reconstructive vascular procedures. Specifically, a graphite coated latex graft with an associated power source is used as a vascular substitute. The results obtained were extremely encouraging in that the latex vascular prosthesis proved to be as effective as homograft material in maintaining an intact circulatory system upon implantation. The body of the thesis deals with the electrostatic theory of biological membranes, the experimental procedure, results and suggestions are presented for further work in the field of synthetic vascular prostheses.

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Chapter 1

COAGULATION

1.1 PHYSIOLOGICAL ASPECTS OF INTRAVASCULAR CLOTTING

Despite, or perhaps because of, advances in medical knowledge, the problem of thromboembolism is as important today as it ever has been. Whether intravascular clotting is to be considered on the increase or decrease depends to a great extent on whether or not the technological advances in materials design and use in the human body is to be considered relevant. Some of the more recent factors considered to be contributing to the increased occurrence of thromboembolism may be: the increasing longevity of the population in general, the tendency of the surgeon to undertake more extensive exploration, resection, repair and transplantation in all areas of the body, the more involved diagnostic procedures, the extensive therapeutic measures now in use, particularly the battery of new drugs and the widespread use of intravascular infusions of many types and substances, and the advent of bioengineering resulting in the influx of greater numbers of foreign substances being introduced into the body.

More than one hundred years ago it was Virchow who

suggested that the principal factors predisposing to thrombosis are:

- (i) Changes in blood vessel walls.
- (ii) Alterations in blood flow.
- (iii) Physical and chemical changes in the blood.

Except in the matter of detail, most authorities today agree with these precepts (13,71,81).

Changes in blood vessel walls may occur as a result of (30,108):

- (i) Trauma (mechanical, thermal, chemical, bacterial).
- (ii) Degeneration, particularly atheromatous.
- (iii) Nervous influences, such as those seen in Raynaud's disease and spasm of non-specific nature.
- (iv) Other changes occurring in conditions such as thromboangitis obliterans, pulseless disease, polyarteritis nodosa and changes in mast cells, both qualitative and quantitative.

Alterations in blood flow are those primarily of stasis (68,129) which depend upon such influences as gravity, physical activity or lack of it, cardiac decompensation, tumors-benign and malignant, scarring and varicosities.

Physical and chemical changes in the blood include (34,87,119):

- (i) Hemoconcentration resulting from hemorrhage, shock, burns, plasma and serum loss, dehydration and agglutination of solid elements.
- (ii) Alterations resulting from low temperatures (cryoglobulinemia). (Of interest is the low incidence of thrombo-phlebitis in warm climates).
- (iii) Metabolic disorders, tissue destruction, anemias, tumors or inflammation of the pancreas, ingestion of fats and drugs.
- (iv) Protein alterations brought about by intravenous infusion.

1.2 THE PROBLEM

Differences in clotting times of blood or plasma have been studied under a variety of conditions such as:

- (i) After dilution.
- (ii) In the absence of contact with wettable surfaces.
- (iii) After removal of solid elements.
- (iv) With the use of various mechanical devices.
- (v) After addition of anticoagulants.

Variation in clotting and anti-clotting in the thrombotic or postoperative period have been reported by a number of authors. Many of these reports have been conflicting and unanimity of opinion on a method for predicting thrombosis is still lacking (1,2).

It is altogether likely that thrombosis in different patients is not necessarily the result of identical changes in their broad respective coagulation mechanisms. It is probable that these changes occur as a result of alterations in a number of factors in each instance and that while some of the latter may be basically the same in all patients, they may be of a different nature in others.

There is no reason to believe that, in the majority of instances, the chemical changes in the blood may be of greater importance than alterations in the vessel walls or rate of blood flow. This belief is of course predisposed if a segment of vessel wall is replaced with an alloplastic material. It is also likely that these chemical alterations include changes affecting various aspects of coagulation such as the formation of thrombin, its inhibition or activation and lastly the overall activity of the fibrinolytic system. It appears likely that blood coagulation is a dynamic process, a limited amount of clotting presumably occurring continuously and, that under normal conditions, various opposing factors prevent the excessive accumulation of the products of this process (5).

The thrombotic tendency is not only important as such, but in certain clinical conditions may actually be responsible for the development of severe hemorrhagic diatheses. These may develop during pregnancy or as a result of shock, burns, surgical trauma, transfusion reactions and malignancy (87,91,95).

1.3 THE MECHANISM OF BLOOD CLOTTING

The protein interactions of blood coagulation as described by Morawitz (74) more than half a century ago is still essentially valid and is shown in fig. 1.3.1. According to this concept, prothrombin, in the presence of thromboplastin and calcium, is converted to thrombin. The latter, in turn, changes fibrinogen to fibrin, the insoluble gel.

With the end of World War II the picture assumed new proportions, as an increasing number of new factors were described, leading to a continually changing and even more complicated mode of action. Despite this apparent confusion, however, the existence of certain "factors" has been sufficiently well documented to warrant a brief discussion of their role in the "clotting mechanism".

It appears now that thromboplastin is formed not only as a result of tissue damage, but may also develop in the blood itself through the interactions of certain plasma proteins and cellular components. Intrinsic thromboplastin was originally thought to result from the interaction of antihemophilic globulin, plasma thromboplastin component (PTC), platelets and calcium (12). This interaction has been shown to occur in several stages (9) and its final product appears to be phospholipoprotein (14). Recent evidence suggests that, in addition to the aforementioned factors, plasma Ac-globulin (factor V) is also essential to the evolution of the final prothrombin-converting principle

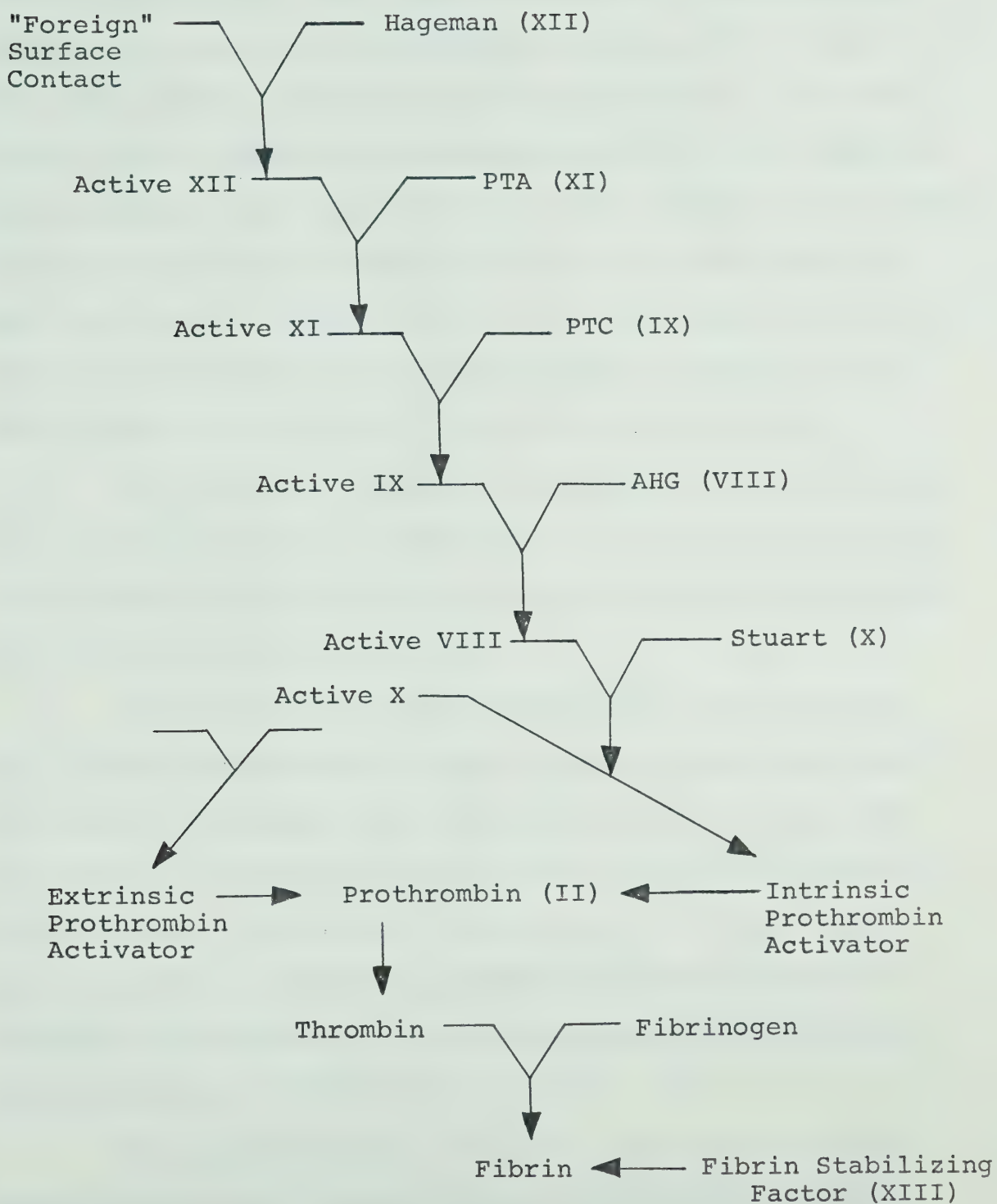


Fig. 1.3.1 (69)

A simplified diagram, showing the protein interactions leading to fibrin formation.

(52). Further implicated in the generation of intrinsic thromboplastin have been plasma thromboplastin antecedent (PTA) (20), factor X (36) and Stuart factor (53). One of the more recently described coagulation factors is proconvertin (SPCA, factor VII, autoprothrombin I) (85). Although it now appears that this factor is not involved in the formation of intrinsic thromboplastin and, although there is some doubt as to whether or not it is essential to the generation of thrombin (4), it apparently is important as an accelerator on the latter's physiological formation.

The action of thrombin on fibrinogen has been shown to be of a proteolytic nature and to involve the splitting off of two or more peptides, followed by the polymerization of this altered fibrinogen (64).

It appears that both thromboplastin and thrombin are rapidly neutralized or inactivated in plasma and there is evidence to suggest that such neutralization may occur by direct inhibition (41,121) as well as by actual destruction of these two factors (33,38,73,117). Of the clot inhibiting factors, heparin probably plays one of the most catholic roles operating at various stages of the coagulation mechanism (15,35,82,123).

As stated earlier, it appears reasonable to consider clotting as a dynamic process including a mechanism for the removal of the final product, fibrin. This fibrinolytic activity can be readily demonstrated in normal

patients and may become excessive in various clinical states. It has been suggested that, as in the case of thromboplastin, the organism also possesses two mechanisms for the production of active fibrinolysin (plasmin) (5). Thus the plasma precursor, profibrinolysin (plasminogen) may be acted on by a tissue activator as well as by an activator formed in the plasma itself.

Various suggestions have been advanced as to the mechanism by which abnormal intravascular clotting is initiated (90,113).

1.4 PHYSICAL FACTORS

Among the physical factors considered to promote intravascular clotting are:

- (i) Injury to the vascular endothelium with the elaboration of a "granular cement" (72), local platelet concentration and the sequence of events set up by such concentration (63,90).
- (ii) Sludging of blood which may occur under a number of conditions such as infection, excessive heat or cold, metabolic disorders and others (60,61).
- (iii) Lack of muscular exercise accompanied by decreased fibrinolysis (11).
- (iv) Lowering of tissue temperatures with cyroprotein precipitation and accompanying

Vascular narrowing (7,34).

(v) Regional body radiation (24,124).

(vi) A positively charged vascular wall (22,40, 93,98,100,102,105-107,109,110,112,125,126).

Of the above factors, which produce intravascular thrombosis, the only one this thesis will deal with is that of charge on the vessel wall.

It should also be noted here that the physical size of the graft also plays an important role in the patency rate of vascular prosthetic implantations. One author (130) states that for long grafts, 40 to 50 centimeters, with an outside diameter of 3.0 to 3.5 millimeters there was an 80% early failure rate and all were occluded by 2 years. Thus the problem of vascular grafting below the waist, where graft size approaches the critical size, has had an extremely poor survival rate.

Chapter 2

BIOPHYSICAL ASPECTS OF INTRAVASCULAR THROMBOSIS

2.1 BIOELECTRICAL FACTORS AFFECTING THROMBOSIS

The study of thrombosis and the associated phenomenon of blood clotting has become increasingly refined and complex since Virchow first summarized the principal factors predisposing to thrombosis. Major efforts have been directed toward the chemical mechanisms which result in coagulation of blood and lysis of blood clots. As a consequence of this chemical orientation, prevention of intravascular thrombosis has been based on the application of a variety of antagonists to clotting factors. This of course, is associated with total systemic alteration of intravascular clotting.

More recently, increasing interest has been directed toward the bioelectrical factors affecting intravascular thrombosis rather than biochemical mechanisms and, as a sequitor, to the application of these bioelectrical principles locally to either produce or prevent the formation of thrombi.

2.2 ELECTRICAL ELEMENTS IN THE VASCULAR SYSTEM

The importance of electrical environment to intra-

vascular thrombosis is predicated on an electrical relationship between the circulating elements of blood and the intimal surface of the blood vessel wall. Conversely, thrombosis associated with injury should be accompanied by an electro-attraction between these two factors.

2.2.1 Electrical Characteristics of Circulating Elements of Blood

Abramson (1,2), in considering the hypothesis that currents of injury are responsible for the leukocyte infiltration in regions of inflammation, demonstrated that, when the estimated current between two electrodes is 0.4 milliamps, the small lymphocytes migrate toward the positive pole with a speed of approximately 15 microns per minute. They move faster than the red cells in the same preparation, and when the current is reversed, they almost instantaneously change the direction in which they have been travelling. This suggests that the lymphocytes possess a negative surface charge.

Abramson (2) also demonstrated that a small electromotive force, in the order of magnitude of that existing in injured tissue, results in the migration of lymphocytes to the positive pole. In subsequent work (3), he demonstrated that red blood cells also migrate to the positive pole. In a series of normal adults, a mean velocity of 1.3 ± 0.02 microns per second per volt per centimeter was established. Completing the spectrum of circulating blood elements, the

surface charge on platelets was determined by microscopic observation of their electrophoretic mobility (10). On application of 5 milliamps of direct current, the platelets also migrated to the positive pole.

The ion groups contributing to the electrokinetic potential on the surface of blood cells have been characterized by several investigators (6,43,67) in an attempt to correlate differences or similarities with their known biological properties. Most authorities have agreed that the major functional components are phospholipid in nature, but that protein and cholesterol may also be present near the surface. Electrophoretic pH-mobility studies suggest that the erythrocyte surface is dominated by strongly acidic groups such as those of a phosphoric acid (43). By making comparisons with known model systems it has been concluded (6) that the dominant group on the erythrocyte was a phosphate, whereas that on a polymorphonuclear leukocyte was a carboxyl. The cation spectra for lymphocytes and platelets were similar to one another, and it was suggested that the dominant groups were both phosphates and amines. Some carboxyl groups may also be present on platelets, and cation bridging between these carboxyl surfaces has been proposed as an important factor in the stickiness of blood cells.

The effect of antisera on the charge of human blood cells has been investigated (26,27), demonstrating that human white cells were negatively charged, but slightly

less so than red cells. Leukoagglutinin treatment of the white cells produced a change of charge, but the change was in the direction of less negativity, in contrast to the increased negative charge seen with the action of anti-red cell antibodies on red cells. The magnitude of the change of charge on white blood cells after leukoagglutinin treatment was about one half that found after treatment of red cells with their antibodies. Human platelets were found to possess negative charges approximately four times as great as those of red cells or white cells, but the possible effect of anti-platelet antisera on normal platelet charge has not, as yet, been investigated.

2.2.2 Electrical Characteristics of The Blood Vessel Wall

Direct determination of the electrical characteristics of blood vessel wall was first performed by Sawyer and Pate (106) in 1953. The normal aortic wall was found to have an electrical potential difference in that the aortic intima was normally negative with respect to the adventitia. The magnitude of this potential difference was found to be 1.5 millivolts. More recently, an active transport of ions by the blood vessel wall has been demonstrated. It has been suggested that this transvascular ion concentration may affect electrical charge. It has been hypothesized that the normal blood vessel wall performs work in order to maintain transmural potential differences by means of the active transport of ions (104).

In this manner, minor injuries are prevented from resulting in the chain of events terminating in intravascular occlusion.

2.3 ELECTRICAL PHENOMENA AT BLOOD-INTIMA INTERFACE

It has been suggested (120) that the forces of attraction and repulsion between the vascular intima and the formed elements of the blood cannot be divorced from those existing within the cell membrane, thus it is appropriate to start with a survey of the phenomenology at a cell boundary as a whole, as shown in fig. 2.3.1.

The nature of the distribution of charges at the solid-liquid interface giving rise to the potential difference associated with electrokinetic phenomena was first considered by Helmholtz in 1879, who introduced the concept of the electrical double layer (17). Two electrically charged layers of opposite sign were assumed, one fixed at the solid surface and the other close to the surface layer in the mobile liquid phase. This is shown in fig. 2.3.2. The charge on the surface layer is exactly balanced by an equivalent number of oppositely charged ions in the liquid layer since the double layer as a whole must be electrically neutral. Within the double layer the potential drops to zero, and the rest of the liquid phase is electrically neutral since it must contain an equivalent number of positive and negative ions. It has also been suggested (17) that the two layers could be considered as being equivalent

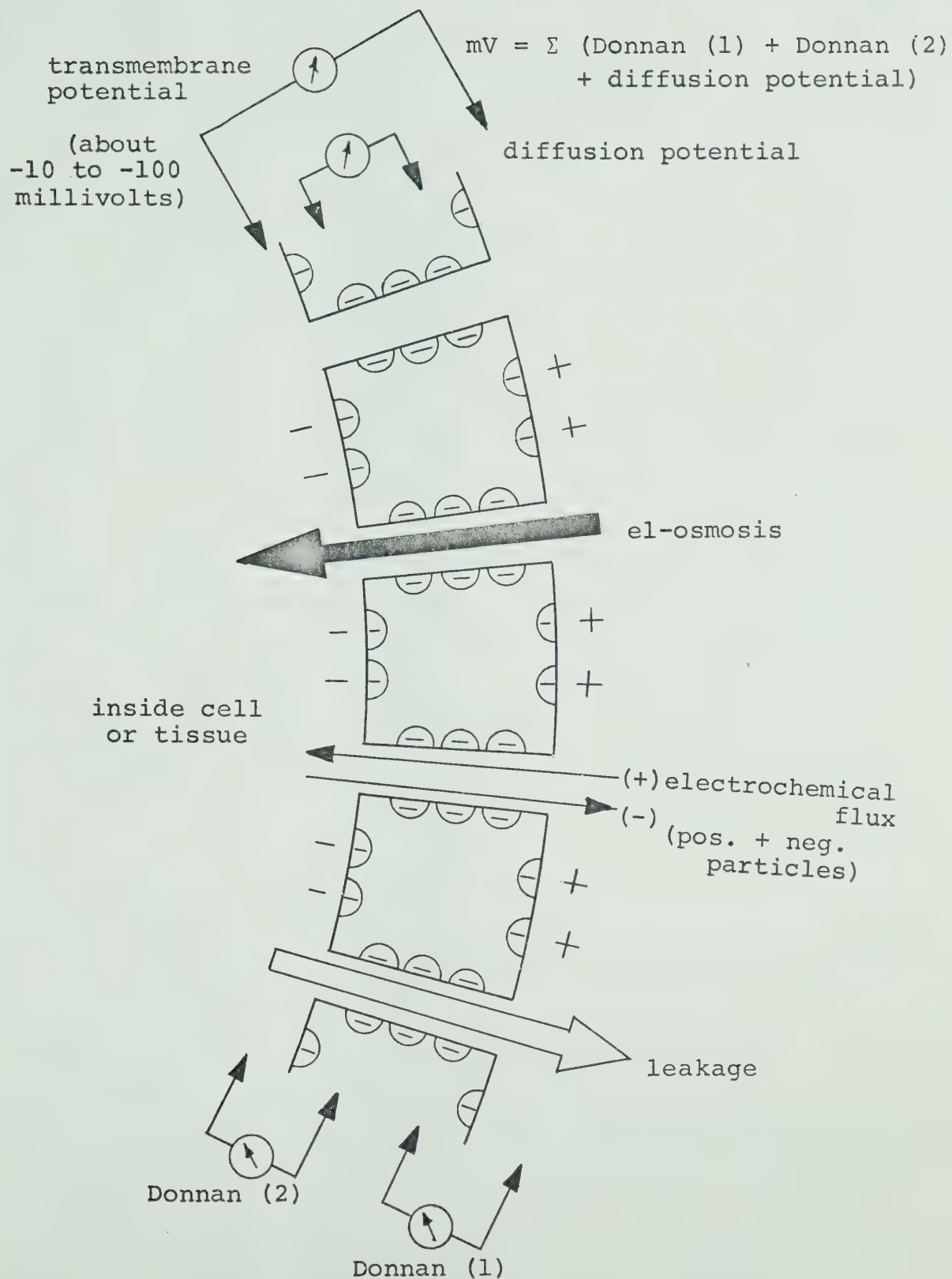


Fig. 2.3.1
Summary of the electrical events
in a cell wall (schematic)

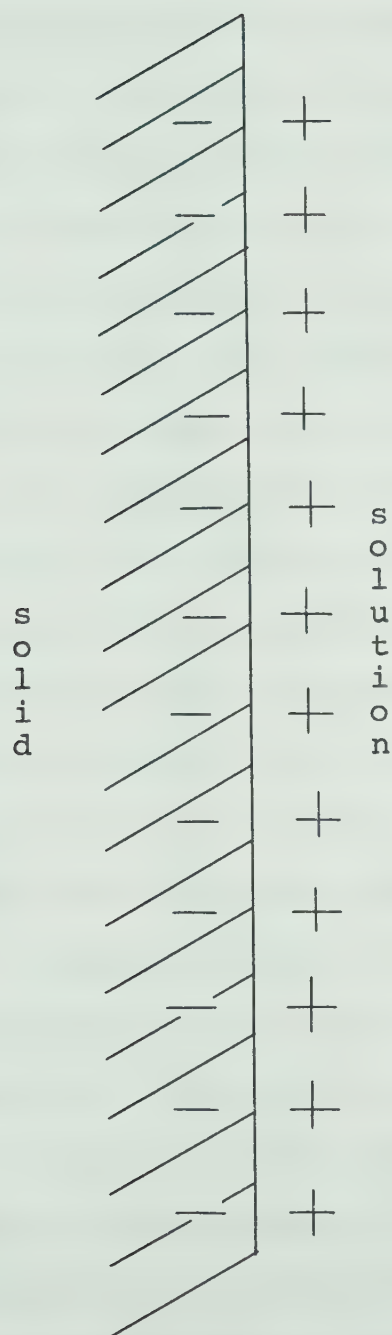


Fig. 2.3.2

The simple picture of the electric double layer.

to a parallel plate capacitor.

This simple picture of the double layer, however, does not take into account the thermal motion of ions in solution, which would prevent the formation of such a compact arrangement. This can be seen if a plane surface uniformly covered with an aqueous solution containing positive and negative ions is considered. The positive ions will be attracted by the surface charge and tend to concentrate near the surface, thus reducing the electric field intensity, due to the surface, at points further out in solution. Hence, the concentration of positive ions will decrease, rapidly near the surface and more slowly further away. Negative ions will be repelled by the surface and thus their concentration will be reduced, particularly in the vicinity of the surface. The net result is that close to the charged surface there will be an excess of positive over negative ions, the difference decreasing with distance until the point is reached in bulk solution where there is an equivalent number of positive and negative ions to maintain electroneutrality. The effect of thermal agitation is, therefore, to create a diffuse double layer (120), with the total net charge in the solution balanced by the equal and opposite charge on the surface. The potential Ψ within the diffuse double layer decreases with distance from its value, Ψ_0 , at the surface, rapidly at first then more and more slowly until it is zero in bulk solution. This picture of the diffuse double layer is illustrated in fig. 2.3.3.

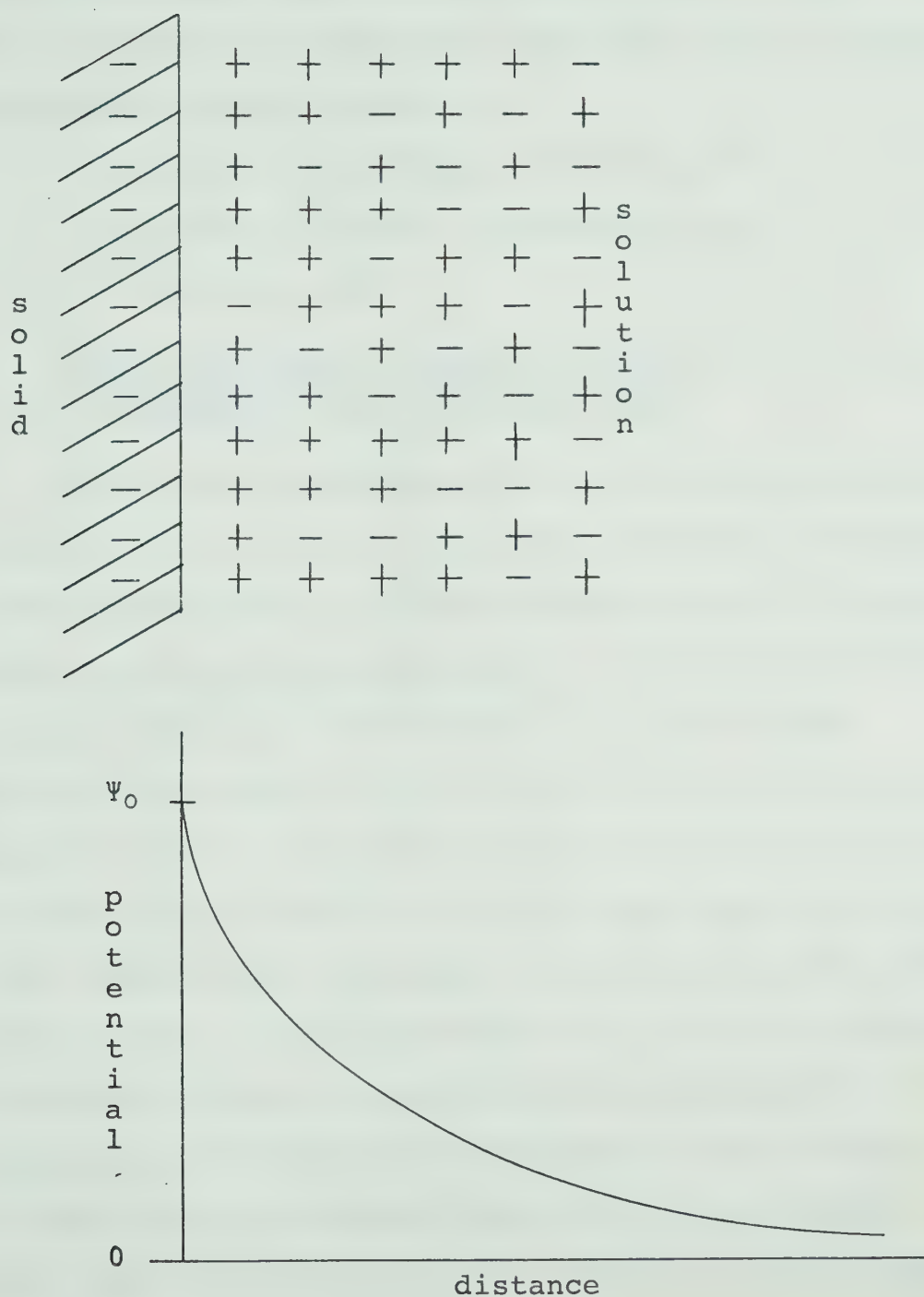


Fig. 2.3.3 (69)

Schematic representation of the diffuse double layer and the decay of potential with distance from the plane surface.

There are two main factors which will have an effect on the ionic distribution and the decay of the potential in a system as described previously, which pertain to the vascular system, these are:

- (i) Effect of surface potential (69).
- (ii) Effect of ionic concentration and valency (69).

2.3.1 Effect of Surface Potential on Ionic Distribution and Potential Decay

For a given concentration of ions of given valency, the shape of the potential decay curve is fixed, since at any given concentration of ions the decay curve is solely dependent on the surface potential ψ_0 . The origin of the curve is determined by the value ψ_0 . At high surface potentials of the order of 100-150 millivolts (127), the rate of decay of ψ over a particular region close to the surface is much greater than when ψ_0 is low. The reason for this is that the negative ions are almost completely expelled from the region close to the surface when the potential is high and the concentration of positive ions in this region is correspondingly increased, the structure of the diffuse double layer being determined essentially by the ions of the opposite sign to the charge on the surface. This is generally the situation in colloidal systems.

2.3.2 Effect of Ionic Concentration and Valency On Ionic Distribution and Potential Decay

An increase in concentration of the ions in solu-

tion or change of pH in the vascular system will, at constant surface potential, mean a proportional increase in their concentration near the surface, due to lack of repulsive forces, resulting in a faster rate of decay of potential. The distance to which the diffuse part of the double layer extends from the surface is also reduced, that is the double layer is compressed. Increase in valency will have the same effect with increased attraction with the positive ions and increased repulsion with the negative ions, although at high surface potential the latter is unimportant.

Potential decay curves illustrating these effects are shown in figs. 2.3.2.1 and 2.3.2.2.

2.4 MODERN CONCEPT OF THE DOUBLE LAYER

To simplify the mathematical treatment of electrokinetic phenomena, it is useful to consider, as Helmholtz postulated (16), that all the ions in the diffuse double layer are located in a single plane at a distance "d" from the surface. The diffuse double layer is now equivalent to a parallel plate capacitor and called the Helmholtz double layer (16). The distance "d" is called the thickness of the double layer and is related to the concentration and valency of the ions in solution by:

$$d = \sqrt{\epsilon K T / 4 \pi e^2 \sum n_i Z_i^2} \quad (19)$$

The only interaction so far considered between the charged surface and the ions in solution is due to electro-

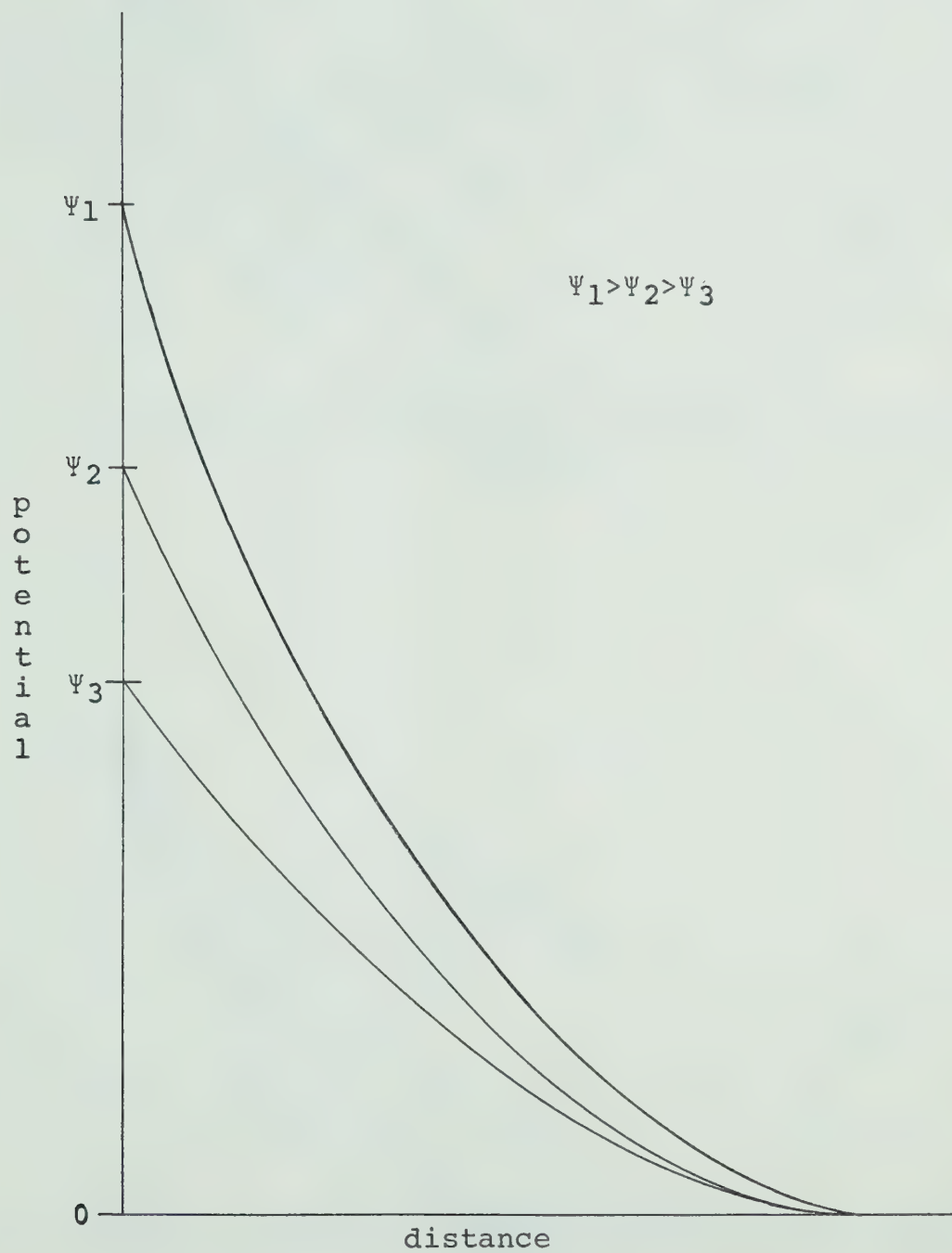


Fig. 2.3.2.1

Effect of surface potential
upon the decay of potential

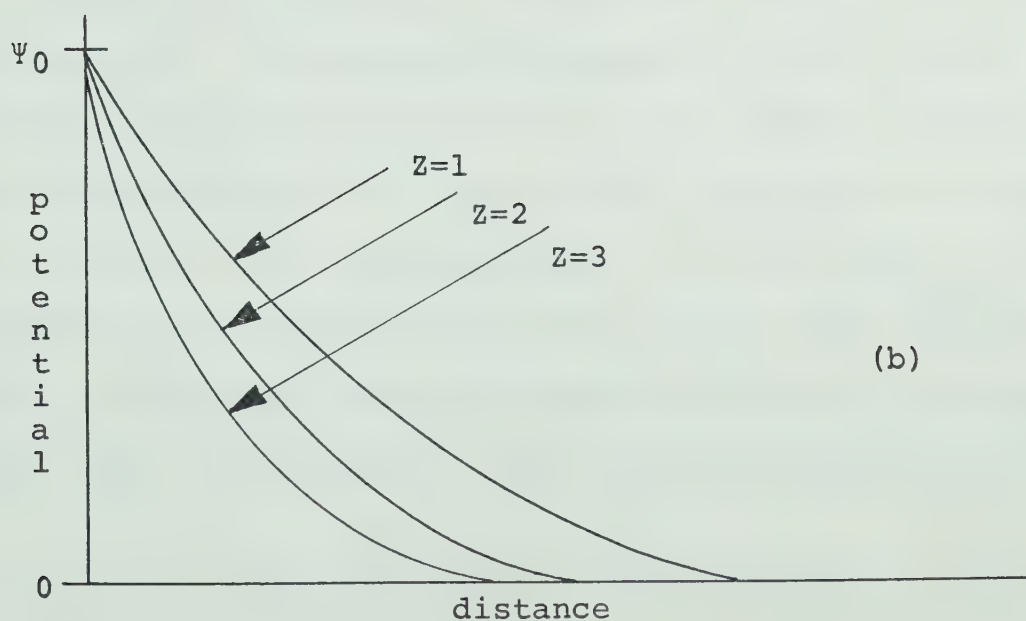
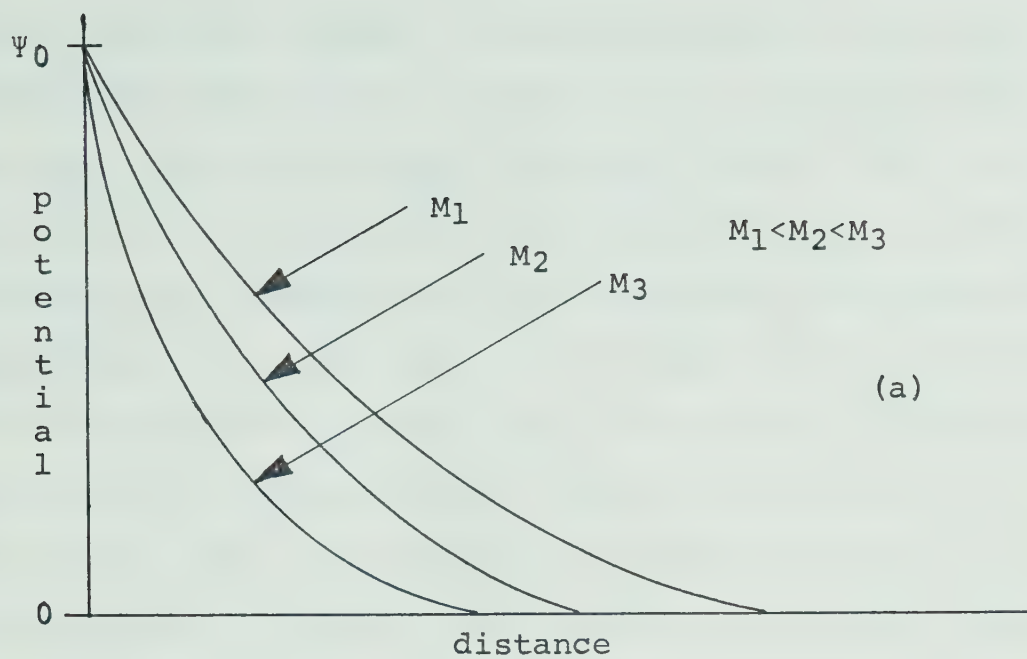


Fig. 2.3.2.2

(a) Effect of concentration of univalent ions upon the decay of potential

(b) Effect of valency Z upon the decay potential

static forces and normal thermal agitation is sufficient to overcome these forces. Stern (46,69), suggested that the region near the surface should be divided into two parts, the first a compact layer adjacent to the surface containing any adsorbed ions and the second consisting of the diffuse Gouy layer (46). The compact part of thickness δ is called the Stern layer and the potential at the boundary between the Stern and Gouy layer is designated $\Psi\delta$. In this theory Stern also allowed for the dimensions of the ions which had, hitherto, been considered as point charges.

In addition to any ions that may be adsorbed, the surface will be solvated by a layer of solvent molecules, probably of the order of one molecule thick, as a result of the attractive influence of the surface charge on the water dipoles (127). The boundary between the rigidly held solvation layer and the solution is the surface of Shear (19,46) associated with electrokinetic phenomena, and the zeta potential (ξ) (19,69,103,120). In most cases ξ will be less than $\Psi\delta$ (unless large ions are adsorbed) and part of the diffuse layer will be located within the solvation layer. This modern double layer theory is shown in fig. 2.4.1.

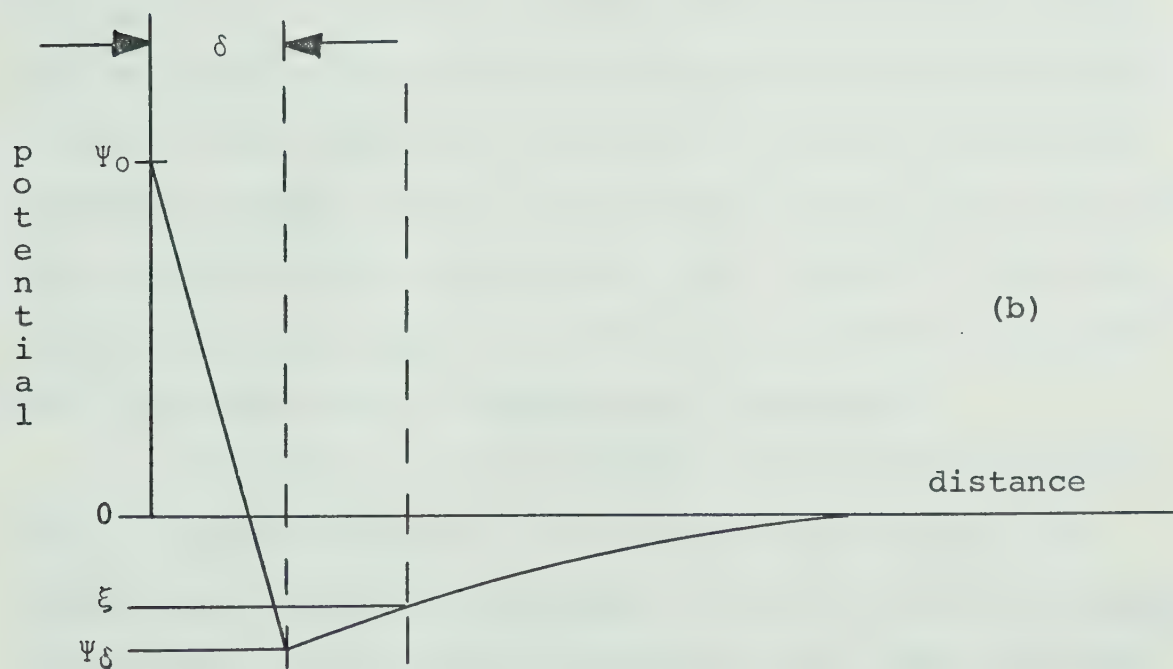
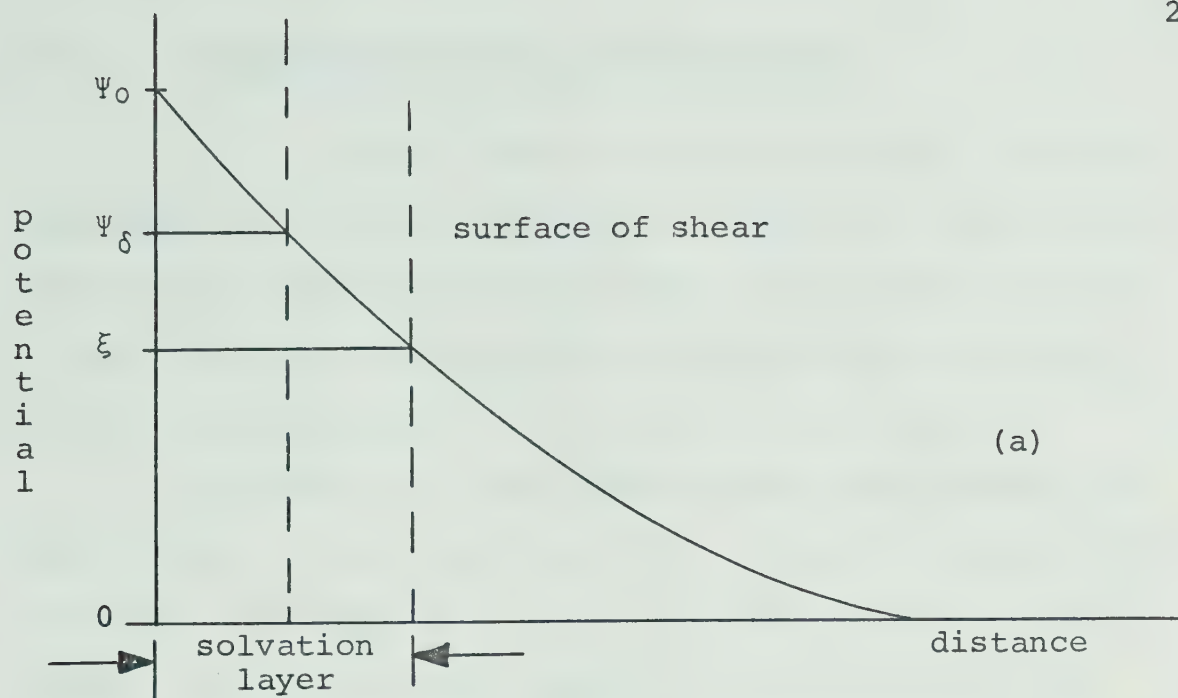


Fig. 2.4.1
Potential decay curves for a
double layer at a plane surface

- (a) $\Psi_0 > \Psi_\delta > \xi$ part of the diffusion layer
in solvation layer
- (b) $\Psi_0 > \xi > \Psi_\delta$ but with a reversal of sign of
 Ψ_δ & ξ due to a strong
adsorption in the Stern layer

2.5 APPLICATION TO THE VASCULAR SYSTEM

In the case of a narrow cylinder, as seen from appendix II, the electrostatic forces on the formed elements, in this simplified scheme, are towards the axis of the cylinder, resulting in repulsion of the formed elements from the surface to the axis of the cylinder.

Overbeek (56), has schematically represented the force fields as shown in fig. 2.5.1. Here the total of the attraction and repulsion curves give the effective total energy. In this case the attractive forces are due to London-Van der Waal forces (120) and the repulsive forces are due to the electrokinetic effects previously described. It can be seen from fig. 2.5.1 that if the electrokinetic effect was reduced there is a critical value Ψ_c at which the total energy becomes attractive. This effect is the basis for onset of agglutination of the blood elements and eventual occlusion due to intravascular thrombosis.

This idea of total energy proposed by Overbeek is also of prime importance in discussing the concepts of stickiness, wettability and other areas of interest related to materials designed for interfacial use. These concepts are therefore dependent on the theoretical views on precipitation of colloidal solutions.

2.6 ALTERATION OF BLOOD VESSEL WALL ASSOCIATED WITH INTRAVASCULAR THROMBOSIS

Sawyer and Pate (106) initially demonstrated that injury to a blood vessel can vary or destroy the normal

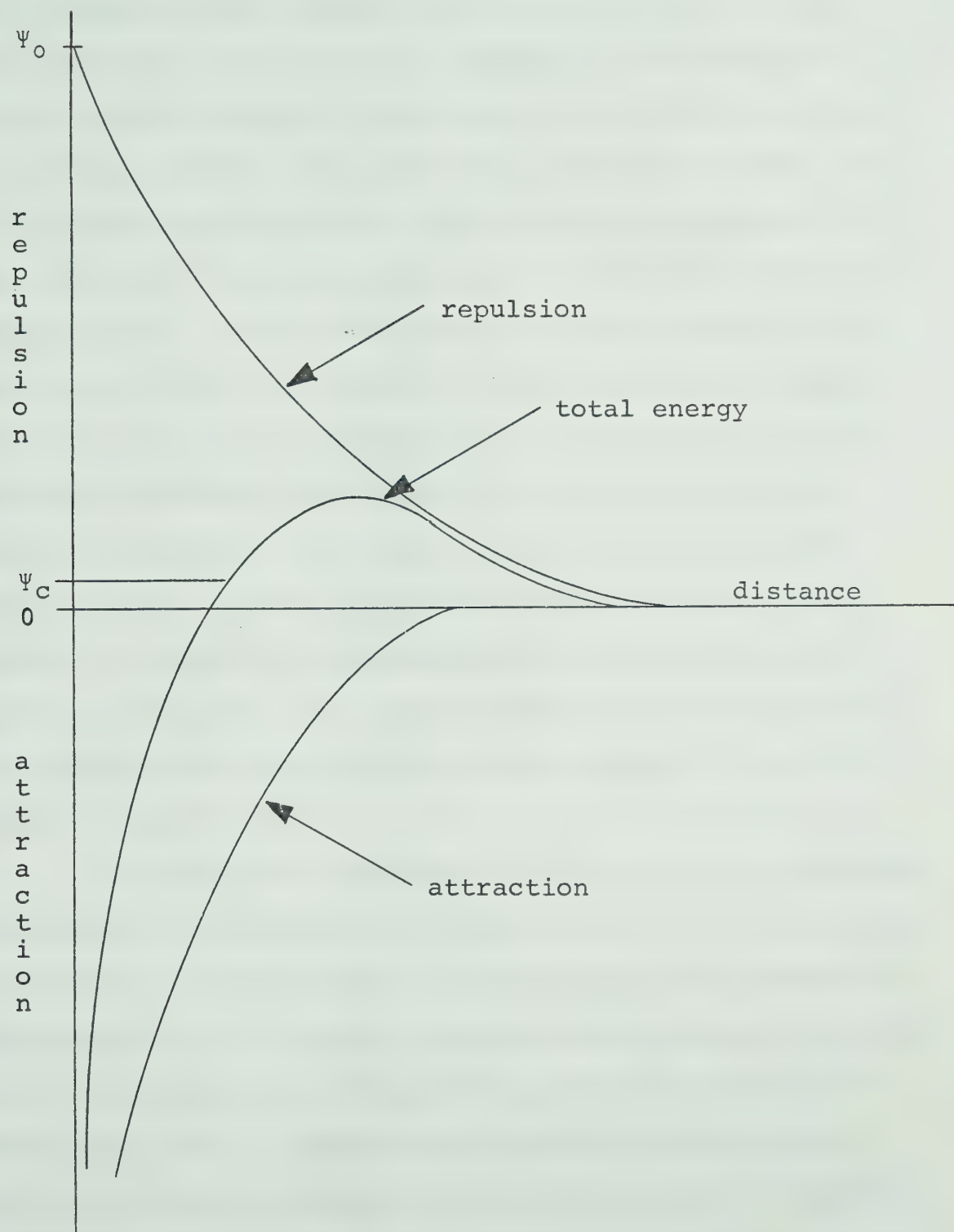


Fig. 2.5.1 (56)

The interaction between attraction
and repulsion near a boundary

transmural potential difference, the intima often becoming highly positive with respect to the adventitia. It has also been shown (105) that the passage of an electrical current across a normal blood vessel wall would precipitate a thrombosis beneath the positively charged electrode. If this current persisted for a sufficiently long period of time, complete occlusion occurred. A subsequent study (107) indicated that, after transection or severe crushing of a blood vessel wall, the intima of the injured area became relatively positive with respect to the adventitia. Calculations suggested that the flow of current, which a potential difference associated with injury can produce through tissue, closely approximates the measured injury current in traumatized and subsequently thrombosed blood vessels. The values also approximated the current per unit area, which, when applied artificially, produced thrombosis (93,107,109,110,112).

Histological evaluation of the reaction of vascular endothelium to injury has afforded presumptive complementary evidence of the importance of bioelectrical environment of blood vessels (72). Normal vascular endothelium is composed of flattened pavement-like cells separated by argyophilic cement lines (56). Immediately beneath the endothelium there is a layer of homogeneous material which, on staining, indicated the presence of acid mucopolysaccharides. In the human vascular system, mast cells are most numerous in the intima and connective tissues, core of heart valves and fairly numerous in the intima of coronary arteries, but

they are rare in other arteries (56). In veins, they are only occasionally demonstratable (56).

When a blood vessel is occluded and stasis is produced, focal groups of cells display argyrophilic granules around their nuclei, and coarser granules also appear within the cells near the cement lines. At the same time, argyrophilic circles appear on the cement lines and within the endothelial cells enclosing small bubbles of meta-chromatic material. Complete stripping of the adventitia from the veins interferes with the capacity of the endothelium to form cement. Endothelial granules are less common and the confluent cement is thinner and less meta-chromatic than when the vessel is only partially deprived of its endothelium. In areas where there is insufficient material for adequate confluence and the cement is poorly formed, leukocytes and platelets accumulate and microscopic thrombi result. Chemical injury results in the same changes (56).

In heparinized animals, foci of reticulated cement is still formed when the adventitia is stripped from the vein. If this reticulated cement is well formed and covers the injured area, no thrombosis occurs. In contrast, protamine prevents adequate coalescence and permits the formation of minute thrombi after partial stripping of the adventitia from the vein. It is inferred that the combination of meta-chromatic substance and granules of cement is hindered by the drug, protamine. Because protamine is an antagonist to heparin it is thought that the meta-chromatic substance contains heparin (56).

The meta-chromatic component is not a product of the endothelial cells, but probably comes from the mast cells. When the injured area becomes covered by a gelatinous film of cement containing heparin, which has a strong negative charge, the tendency to become positively charged is counteracted. Inadequacies of the natural heparin production predisposes to the attraction of cellular elements of blood to segments of severely traumatized blood vessels (56).

2.7 ELECTRICAL PREVENTION OF THROMBI

Sawyer and Deutch (100,101) demonstrated that a current imposed upon a segment of blood vessel would delay intravascular thrombosis for periods up to 12 hours, if the negative pole of the source was attached to the intima. These authors also showed that with currents above 0.3 milliamps, thrombosis in the crushed femoral and carotid arteries occurred less frequently. Engler and co-workers (40), considering the same possibility, anastomosed short arterial homografts bilaterally into the femoral arteries of dogs. Insulated hemicylindrical electrodes were placed around each homograft, and 250 volts were delivered from the negative pole of a D-C voltage regulator to the electrodes on one artery alone, the opposite artery serving as a control. The rate of thrombosis was identical for the two sides. However, the induced electrostatic field was maintained for only 6 hours, and the vessels were studied a week later. Although these results are not very encour-

aging, other groups (93,112) have used a platinum foil wrapped around the femoral veins and a negative current of 1 to 5 milliamps to prevent thrombosis after injection of topical thrombin, thus demonstrating that negative electric currents may prevent chemically induced thrombosis.

These authors (93,112) also demonstrated that mechanically induced thrombosis may be prevented or markedly reduced by applying a negative current of 3-4 milliamps to stainless steel arterial conduits. The patency rate after 72 hours was 9 out of 11.

Other reports concerning the ability of a negative current to repel clot formation may also be cited. Sadd and associates (98) inserted steel and silver coated rings in the inferior vena cava and applied a small negative potential, 2 volts, to half of the preparations. The negative charge significantly decreased the incidence of thrombotic occlusion of both the steel and silver coated rings. In a subsequent study (45), methyl methacrylate rings were coated with graphite, inserted in the inferior vena cava, and either negatively or positively charged from a 3 volt source. All positively charged rings demonstrated thrombotic occlusion, while the negatively charged rings were patent and only occasionally contained minute thrombi. Winfrey and Foster (126), demonstrated that a short linear arteriotomy of 6 centimeters resulted in thrombosis in a vast majority of cases. However, when a negative charge was applied to a wire suture, more than 80 percent of the vessels remained patent. Finally, Carey and Lepley (22)

presented histological evidence that a continuous direct electrical current from a negative pole resulted in apparent inhibition of inflammatory cell infiltration in the region of the electrode.

Chapter 3

DEVELOPMENT OF THE PROSTHESES

3.1 THE PROSTHESIS

The prostheses used during the experimental portion of this exercise can be divided into 3 basic groups:

- (i) Plain latex.
- (ii) Latex with a colloidal graphite inner lining.
- (iii) Latex with a colloidal graphite inner lining with a mercury cell attached.

3.2 LATEX AS A VASCULAR PROSTHESIS

Because of its availability, rubber was the first synthetic material used for the grafting of blood vessels (51). As early as 1915, Horsley (51) described the use of rubber tubes to replace segments of the aorta in experimental animals. He was impressed with the sturdy layer laid down around the tubing and of its close resemblance to adventitia. With this in mind he theorized that the rubber would eventually degenerate, leaving a strong fibrous tube to support the arterial flow. Although such an end point was never obtained, he did mention in his report (51), that there was a slow clot formation within the tube with the longest success remaining patent for six months.

Early in the clinical investigation of vascular

disease, it was discovered that healing at the suture lines would take place even in the most diseased vessel (18). Regardless of the grafting material inserted, replacement in the large vessels, such as the aorta, had been highly successful. Because of the lower flows and lower pressures, the problem, as it pertains to vessels less than eight millimeters in diameter, is entirely different (114). Edwards (37) has stated "that the loss of elasticity is the most important cause of graft occlusion" in the smaller arteries. With this in mind, it is evident that the ideal vascular substitute should have the proper elastic characteristics and that it should maintain these characteristics for a prolonged period of time. Besides being important in maintaining diastolic blood pressure, the property of elasticity allows for the transmission of a normal pulse wave (80). To date, all presently acceptable vascular grafts are soon reduced to rigid tubes in situ (79).

Studies of the normal arteries in humans and in animals reveal that the smooth muscle and elastic tissue network responsible for the arterial retractile force over a wide range of distension shows mechanical and thermal properties often associated with those found in natural rubber (51,65). Since the search for the ideal vascular substitute continues and elasticity appears to be an important property of blood vessels, the advantages of rubber as a prosthetic vascular material are believed to be:

- (i) Its close simulation of the mechanical properties of normal vascular tissue.

- (ii) Simplicity of construction.
- (iii) Ease of handling.

3.3 BLOOD-CARBON COMPATABILITY

Very little work has been done on the compatability of elemental carbon and blood with respect to its ability to retard or minimize coagulation. One author (45) suggests that graphite would make a compatible surface, where compatible in this sense means non-injurious to blood and does not promote the coagulation process, since it is elemental carbon and thus relatively non-reactive. Other reasons cited in favour of carbon as a blood interface are its extreme non-wettability, its conductivity, its inertness and its lubricative properties (45). Graphite is elemental carbon in the form of laminar hexagonal crystals, it is a non-metallic conductor and has been used extensively in electrical circuits. In the colloidal form extremely fine particles of graphite are suspended in a carrier. The particles are separated for the most part as crystal aggregates and the mass mean particle size is between 1 and 2 microns. There are a very few flocculent particles present, ranging in size up to 8 microns in diameter.

3.4 PRODUCTION OF THE PROSTHESIS

All prostheses were constructed by dipping various dowel sizes into a liquid latex and catalyst; twice for grafts to be used in the arterial anastomosis and once for

venous prostheses. This was an empirical determination and the difference in dippings was an attempt to mimic the elastic properties of both vein and artery without the graft becoming excessively rigid. This procedure produced venous grafts with a wall thickness of 0.5 millimeters and arterial grafts with a wall thickness of 1 millimeter. The use of various dowel sizes was to ensure that the grafts were the correct diameter for anastomosis since one of the biggest problems in using latex is to have the ends of the prosthesis the precise diameter of the severed vessel to produce a suture line which will not "wrinkle" either graft or associated vessel which would allow excessive bleeding.

The materials used in all the prostheses was latex* and colloidal graphite in the form of Electrodag 199†. As stated earlier, latex was used for its availability, elasticity, ease of graft production and its relative inertness. Electrodag 199 was chosen as the conductive coating to be applied to the prostheses since it is a colloid, is flexible and cures at 110°C in approximately 15 minutes.

All the prostheses used were 2.5 centimeters in length and varied from 4 to 9 millimeters in internal diameter. The graphite was applied to the latex by washing the finished latex tube with Benzene and pouring a solution of

*Latex - International Latex Corporation, Dover, Delaware.

†Electrodag 199 - Acheson Colloids Limited, Brantford, Ontario.

Benezene-Electrodag 199 in the ratio of 4:1 by weight into the graft, thus allowing the total internal surface to be coated with the solution. The excess graphite was then poured out of the graft and the finished prosthesis was cured at 110°C for 20 minutes in an oven. The graphite applied in this manner produced an even coat which covered the entire surface, giving an impedance of approximately 14 ohms per square millimeter. In the case of the prostheses with battery attached, the mercury cell was first connected to 20 centimeters of phonograph lead-in wire, one wire for each terminal and then coated in epoxy, to seal it from the surrounding tissue. The wire connected to the negative pole of the mercury cell was then bared to a length of 2 centimeters and woven through the latex tube as shown in fig. 3.4.1. This was accomplished by passing a 21 gauge needle in and out of the latex tube and then withdrawing the needle after the wire was introduced into the lumen of the needle. After the wire was thus woven into the latex tube, a layer of latex was placed over the exposed wire on the external side to insulate it. The graphite was then applied in the same manner as the plain graphite grafts.

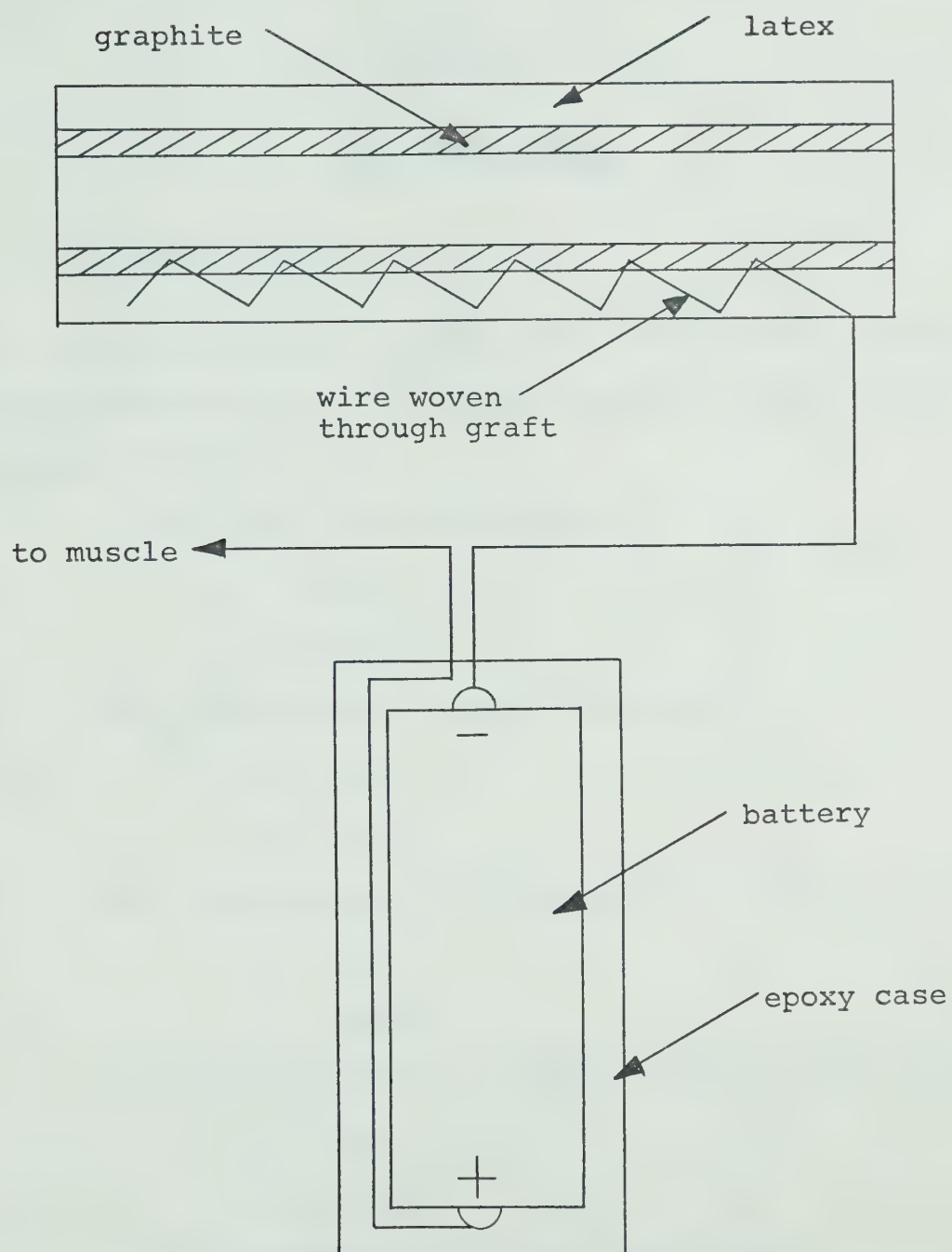


Fig. 3.4.1
Construction of prosthesis
with battery attached

Chapter 4

IN VIVO TESTING

4.1 SURGICAL METHOD

The experimental schema was broken down into two separate stages with the following groups in each stage; also shown in Table 4.1:

- (i) End to end anastomosis,
 - a) vein
 - b) artery
- (ii) Implant of charged latex graft,
 - a) vein
 - b) artery
- (iii) Interpolation with reversal of ends,
 - a) vein
 - b) artery
- (iv) Implantation of graphite coated latex graft,
 - a) vein
 - b) artery
- (v) Implantation of pure latex grafts,
 - a) vein
 - b) artery

The finished vascular prostheses were inserted into

the ilio-femoral region in either vein and/or artery in a random selection of 10 adult mongrel dogs, male and female, in good health, weighing 11 to 15 kilograms. The surgery was done under aseptic conditions only in the case of the end to end, interpolation and charged latex prostheses since the others were all predetermined to be acute experiments.

All experimental animals were anaesthetized, initially, with intravenous Pentobarbital Sodium (30 milligrams per kilogram) and maintained in a state of anaesthesia with successive doses of Sodium Pentothal as required.

The anastomosis site was exposed and the vein and/or artery freed from the surrounding tissue. The transmural catheters were then positioned, as shown in fig. 4.1.1 and the preoperative tests conducted. The vessel was then prepared for the anastomosis in the following manner:

(i) Group I - end to end anastomosis.

The vessel was stripped of its adventitia for a distance of 5 millimeters on either side of tip of the internal catheter and transected. The ends of the transected vessel were then sutured together using a multiple suture technique with a continuous stitch of 5-0 mersilene suture on a V-7 needle. This suturing technique was used on all of the experimental subjects. Immediately postoperatively the series of tests were rerun.

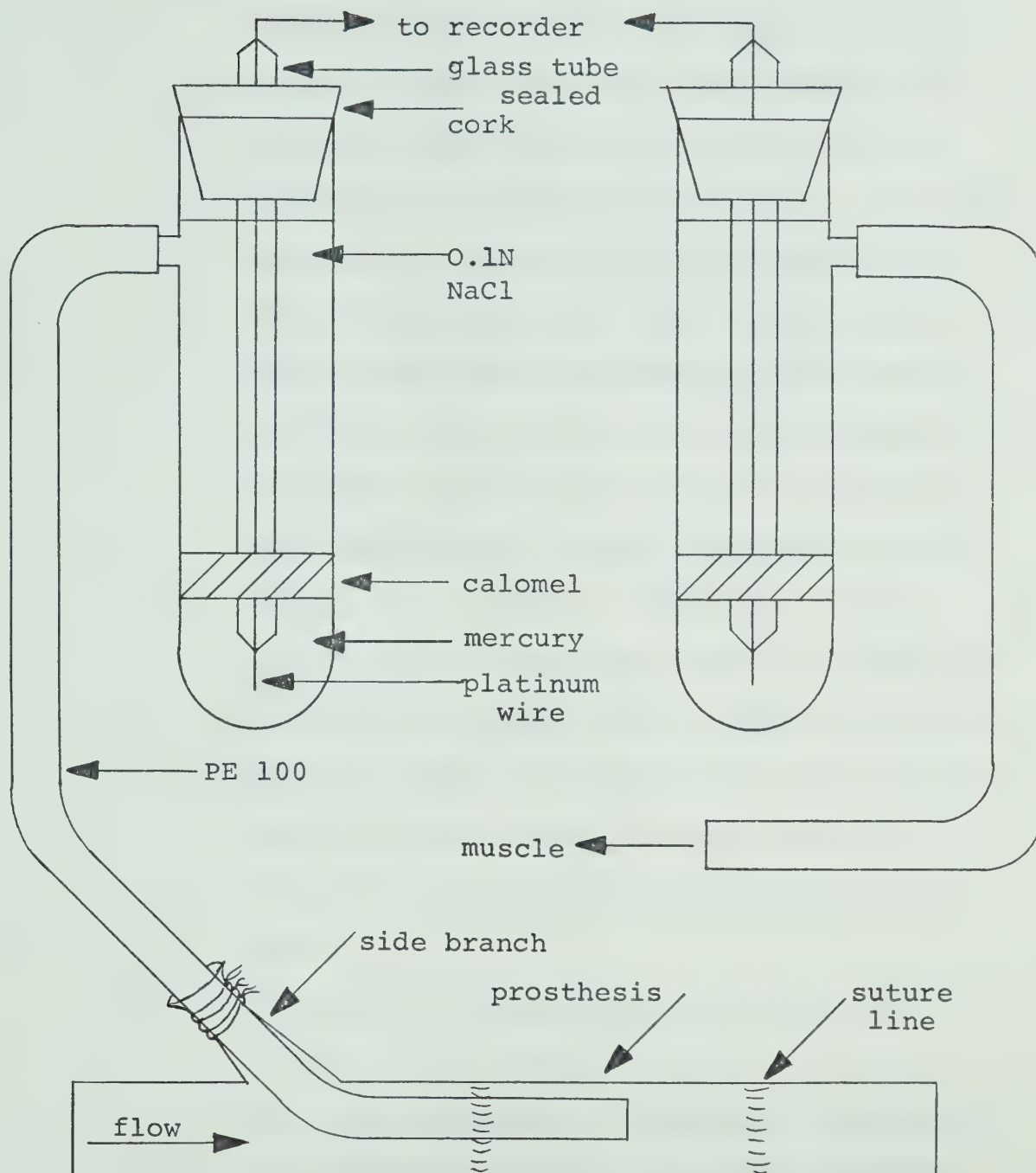


Fig. 4.1.1

Schematic of calomel Half-cell connection
for transmembrane potential measurement

(ii) Group II - charged latex graft.

In this case a segment of vessel, the exact length of the prosthesis, was removed and the graft then sutured in place using the aforementioned suturing technique. For this group the positive pole of the battery was not sutured into place until immediately after blood flow had resumed in the vessel. This was done to give the formed elements of the blood time to agglutinate at the suture line and possibly form a haemostatic plug before the current was applied. In this manner it was hoped that excessive bleeding could be eliminated before repulsion of the formed elements occurred due to the current being applied. After bleeding had been controlled the postoperative tests were initiated.

(iii) Group III - interpolation with reversal
of ends.

With this procedure a segment of the vessel was removed and reinserted in an opposed position. Again, after bleeding was considered to be under control the postoperative tests were begun.

(iv) Group IV - graphited latex.

This procedure followed the same methodology

as the group II anastomoses with the exception that the battery need not be connected.

(v) Group V - latex.

This implantation followed precisely the same method as group IV.

As has been mentioned, in all cases a measurement of transmural potential preoperatively as well as immediately postoperatively was undertaken. In the acute experiments, two calomel half-cells were used as nonpolarizing electrodes. The negative calomel half-cell was attached to a polyethylene catheter, filled with 0.1 N NaCl, which was passed through a side branch of the vein or artery to a point in the vessel estimated to be approximately midway through the prosthesis after completion of the anastomosis. The positive calomel half-cell was connected in a similar manner to a muscle in the vicinity of the anastomosis. This apparatus, used to measure the transmural potentials, is shown in fig. 4.1.1.

For the long term experiments, an open lumen, small diameter polyethylene catheter (PE 100) with an internal diameter of 0.86 millimeters was passed through a small side branch of the vessel under experimentation and tied in place so that the tip of the catheter would eventually be in the middle of the prosthesis, this was filled with 0.1 N NaCl and capped on the external end to prevent escape of the blood. Another catheter was sutured into a muscle close to the site of anastomosis, this electrode was similarly

filled with 0.1 N NaCl and capped on the external end. At the time of transmural potential measurement, these electrodes were connected to their respective calomel half-cells and measurements taken. During the time the animals were not being tested, the catheters were sutured into a subcutaneous pouch so that the tips of the catheters could not be torn out and yet be accessible by a simple skin incision at the time of transmural potential measurement. The open lumen catheter inside the vessel also substituted as a catheter to obtain blood samples for clotting time, pH measurement, platelet count, prothrombin time and fibrin formation tests.

When taking potential measurements, the outputs of the calomel half-cells were connected to a Beckman type 9806A coupler in a Beckman type R Dynograph recorder. The AC coupler was used in the DC mode with frequency response at a minimum, Hi. freq. =1, and the response switch on average in order to delete any extraneous signals impressed on the input leads.

The transmural potential, along with the other tests, were measured preoperatively, immediately postoperatively, then every day for 3 days and at weekly intervals thereafter until the graft occluded or the animal was sacrificed.

Clinical patency was determined preoperatively, immediately postoperatively and at each succeeding test by palpation and with the use of a per-cutaneous doppler flow

probe. This method proved to be an extremely dependable method of determining whether there was or was not flow in the vessel, since only qualitative and not quantitative results were required. In the first experiments used to develop the technique of implantation, potential measurements etc., pure latex grafts were used, with the result that the decrease in flow could be detected qualitatively with the flow probe as time progressed. At the time the flow probe registered no flow the graft was removed and examined showing complete occlusion of the lumen in each case. This fact was likewise demonstrated with other prostheses which had occluded during the experiment.

As was mentioned earlier, along with the transmural potential measurements, several other tests were also run, these are:

- (i) pH measurement
- (ii) Platelet count
- (iii) Clotting time
- (iv) Prothrombin time
- (v) Fibrin formation

The pH measurement was undertaken to ensure that the blood was kept at a normal pH (7.37) or alkalotic, since the red cells, leukocytes, fibrinogen and other blood components are known to have a negative charge only with blood having a normal or alkalotic pH (60).

Platelet counts, clotting times, prothrombin time and fibrin formation were used to determine the state of

the coagulation system. This is a rather empirical determination since if the platelet count, and rate of fibrin formation were elevated with the clotting and prothrombin time diminished, it was assumed that the coagulation system was still active, similarly it was assumed that the coagulation system was inactive when the values returned to normal.

TABLE 4.1
Surgical Schema

Group	Type of Anastomosis	No. of Dogs	No. of Anastomoses	No. Occluded	Time To Occlusion	Longest Period of Patency
I	End to End	2	3	0	60 days	60 days ⁺
II	Charged	2	8	2	10 - 17 days	60 days ⁺
III	Interpolation	2	4	1	10 days	29 days ⁺
IV	Graphited	2	6	6	2 - 4 hrs.	4 hrs.
V	Latex	2	6	6	1/2 - 2 hrs.	2 hrs.

⁺ Sacrificed at this time

TABLE 4.2

Transmural Potentials

				Transmural Potential in Millivolts (Normal -10 to -100 mv)																
Group	Dog No.	Site of Anastomosis	Side	Pre Op	Post Op	Days Postoperatively														
						1	2	3	10	17	24	31	38	45	52	59	60			
I	J-589	Artery	R	- 5	- 1	0	- 3	- 8	-10	- 5	- 5	- 4	- 5	- 6	- 5	- 7	- 4			
		Vein	R	- 3	0	+ 1	- 2	- 9	- 6	- 4	- 5	- 3	- 2	- 5	- 4	- 2	- 3			
	J-742	Vein	L	- 4	- 2	- 3	- 1	- 7	- 4	- 4	- 3	- 1	- 2	- 4	- 3	- 2	- 3			
		Artery	R	-10	-59	-57	-57	-56	-50	-50	-50	-48	-50	-50	-45	-46	-45			
II	J-980	Vein	R	- 8	-62	-62	-64	-60	-60	-62	-61	-57	-60	-64	-60	-58	-55			
		Artery	L	- 7	-47	-47	-20	0	+ 1	occluded										
		Vein	L	-15	-63	-61	-60	-55	-62	-60	-60	-60	-61	-58	-57	-59	-60			
		Artery	R	- 4	-58	-58	-57	-62	-59	-57	-55	-54	-56	-58	-60	-57	-55			
	J-925	Vein	R	- 8	-50	-47	-15	+ 3	+ 8	+16	occluded									
		Artery	L	- 6	-76	-70	-54	-68	-63	-63	-65	-63	-60	-55	-55	-50	-50			
		Vein	L	-12	-63	-63	-63	-63	-67	-66	-67	-66	-67	-60	-58	-59	-60			
																		-61		

TABLE 4.3
Changes of pH in Postoperative Days

		pH (normal 7.35)													
Group	Dog No.	Pre Op	Post Op	Days Postoperatively											
				1	2	3	10	17	24	31	38	45	52	59	60
I	J-589	7.39	7.40	7.41	7.40	7.38	7.37	7.36	7.37	7.37	7.35	7.38	7.37	7.37	7.37
	J-742	7.37	7.37	7.36	7.38	7.40	7.36	7.37	7.35	7.37	7.36	7.37	7.38	7.37	7.37
II	J-980	7.42	7.37	7.37	7.35	7.36	7.37	7.34	7.36	7.37	7.38	7.35	7.38	7.37	7.38
	J-925	7.38	7.39	7.38	7.34	7.33	7.32	7.36	7.38	7.37	7.36	7.36	7.36	7.36	7.36
III	J-573	7.40	7.40	7.41	7.40	7.37	7.39	7.38	7.36	7.35	7.37	7.36	7.38	7.37	7.36
	J-628	7.37	7.37	7.37	7.35	7.35	7.40	7.41	7.38	7.38	7.38	7.38	7.38	7.36	7.35
IV	J-624	7.39	7.42	occluded											
	J-488	7.43	7.40	occluded											
V	J-835	7.45	7.38	occluded											
	J-440	7.37	7.38	occluded											

TABLE 4.5

Clotting Times

		Clotting Time in Minutes and Seconds (Normal 5' to 5'30")													
Group	Dog No.	Pre Op	Post Op	Days Postoperatively											
				1	2	3	10	17	24	31	38	45	52	59	60
I	J-589	5'10"	4'30"	4'32"	5'0"	5'1"	5'10"	5'10"	5'15"	5'5"	5'10"	5'8"	5'9"	5'10"	5'10"
	J-742	5'5"	4'22"	4'30"	4'40"	5'0"	5'10"	5'5"	5'8"	5'8"	5'7"	5'6"	5'0"	5'5"	5'8"
II	J-980	5'7"	5'28"	5'20"	5'15"	5'15"	5'10"	5'15"	5'12"	5'20"	5'6"	5'8"	5'7"	5'7"	5'8"
	J-925	5'8"	5'20"	5'20"	5'40"	5'1"	5'0"	5'0"	5'10"	5'6"	5'8"	5'9"	5'8"	5'6"	5'5"
III	J-573	5'0"	4'14"	4'25"	4'25"	4'42"	4'50"	5'10"	5'8"	5'7"	5'10"	5'12"	5'15"	5'8"	5'10"
	J-628	4'59"	4'5"	4'20"	4'25"	5'2"	5'2"	5'0"	5'0"	5'5"	5'6"	5'10"	5'8"	5'5"	5'4"
IV	J-624	5'6"	4'50"	occluded											
	J-488	5'8"	4'35"	occluded											
V	J-835	5'10"	3'20"	occluded											
	J-440	5'7"	3'10"	occluded											

TABLE 4.6

Prothrombin Time

Group	Dog No.	Prothrombin Time in Seconds (Normal 11 - 14 Seconds)													
		Pre Op	Post Op	Days Postoperatively											
				1	2	3	10	17	24	31	38	45	52	59	60
I	J-589	11.6	10.0	9.7	8.9	8.9	9.0	11.1	11.4	11.5	11.3	11.8	11.9	12.0	12.0
	J-742	12.2	9.1	9.0	9.8	10.7	10.8	10.9	10.9	11.0	11.2	11.3	11.3	11.4	11.3
II	J-980	13.0	14.1	14.2	13.6	12.5	13.8	13.6	12.7	13.4	11.3	13.2	12.9	11.0	12.6
	J-925	10.2	10.8	11.0	11.2	10.0	12.2	14.2	14.2	14.0	14.0	14.2	14.3	14.2	14.0
III	J-573	11.0	9.8	9.6	9.8	9.9	10.2	10.2	10.3	11.3	11.2	11.4	11.0	11.0	11.0
	J-628	14.1	11.0	11.1	11.2	11.3	11.2	11.2	11.3	11.4	11.5	11.2	11.3	11.1	11.2
IV	J-624	10.4	9.6	occluded											
	J-488	11.6	9.7	occluded											
V	J-835	12.3	8.4	occluded											
	J-440	12.1	8.6	occluded											

TABLE 4.7
Fibrinogen Concentration

Group	Dog No.	Pre Op	Post Op	Fibrinogen Concentration in Milligram-Percent (Normal 385 - 390 mg%)											
				Days Postoperatively											
				1	2	3	10	17	24	31	38	45	52	59	60
I	J-589	388	310	312	309	301	299	305	376	380	390	390	386	387	387
	J-742	370	306	308	310	345	362	385	390	386	386	387	386	384	382
II	J-980	390	450	470	408	385	380	385	387	390	392	395	390	390	390
	J-925	387	468	469	399	382	385	387	390	388	386	388	386	387	389
III	J-573	392	300	306	310	308	340	376	385	390	391	383	387	385	390
	J-628	375	325	320	315	300	331	387	388	389	387	386	388	387	389
IV	J-624	381	330	occluded											
	J-488	377	351	occluded											
V	J-835	376	320	occluded											
	J-440	380	332	occluded											

Chapter 5

DISCUSSION OF DATA AND SUGGESTIONS FOR FURTHER RESEARCH

5.1 DISCUSSION OF DATA

5.1.1 End to End Anastomosis

The end to end anastomosis was one series of controls to demonstrate that the technique of suturing was practical.

The potential on the suture line of the end to end anastomosis was negative prior to the anastomosis and either zero or less negative than the preoperative measurement immediately postoperatively. Usually, the graft potential returned to near normal between the first and second postoperative day. The potential then stays negative in polarity over the remainder of the period of healing. At one time or another each of these prostheses developed a higher negative potential around the third to tenth postoperative day and returned to normal after this.

The one control subject in this series which developed a positive potential on the first postoperative day, J-589 (V-R) showed mural thrombi along the suture line upon examination. All other experimental anastomoses in this group showed no sign of thrombosis at autopsy.

The pH measurements in this group showed no deviation from the normal range as was expected. The platelet count dropped from a normal value preoperatively to a value, still in the normal range, postoperatively, reaching a low point just on the lower limits of normal on the first day postoperatively, gradually returning to normal on about the tenth postoperative day. This would coincide with the immediate formation of a haemostatic platelet plug and a gradual replenishing of the depleted platelet supply.

As with the platelet supply the clotting times dropped from a normal value preoperatively to a low level immediately postoperatively and gradually returned to normal by the third day postoperatively. This would coincide with the injury to the vessel and initiation of the coagulation process with a return to normal as healing progressed.

The prothrombin time test, which is an indication of the onset of the coagulation system, had a drop immediately postoperatively and reached a low on the second day postoperatively with a continuing increase back to normal on about the seventeenth to twenty-fourth day postoperatively, indicating that the coagulation system had halted at this time.

A similar effect was seen with the fibrinogen concentration. Here there was a decrease immediately postoperatively increasing to normal on the seventeenth and thirty-eighth day. The extended period of returning to normal in the case of J-589 was assumed to be due to having

a positive potential on the first day postoperatively in conjunction with a postoperative transmural potential close to zero with the result that any injury induced an extended healing time.

5.1.2 Charged Graft Implantation

The implantation of the charged-graphited-latex grafts naturally presented a different transmural potential curve than did all the other implants. The potential difference in this case tended to be smoother and much more negative than the normal vessel due to the incorporation of the direct current source. This difference is illustrated in figs. 5.1.2.1 and 5.1.2.2.

The preoperative transmural potentials, ranging from -4 to -15 millivolts, tended to be slightly lower than those found in the end to end anastomosis but were comparable to all other groups. The immediate postoperative, along with continuing measurements, were found to be about an order of magnitude lower than those found in the other anastomoses.

Two of these prostheses were found to be occluded on the seventeenth and twenty-fourth postoperative day. J-980 (A-L) was found to be occluded on the seventeenth postoperative day and examination showed that the wire sutured into the surrounding muscle had broken thus allowing the transmural potential to rise to its final value of +1 millivolt. This occlusion had been suspected with the rising of the potential difference toward zero and on the tenth post-

Fig. 5.1.2.1

Normal transmural potentials of
patent and clotted anastomosis

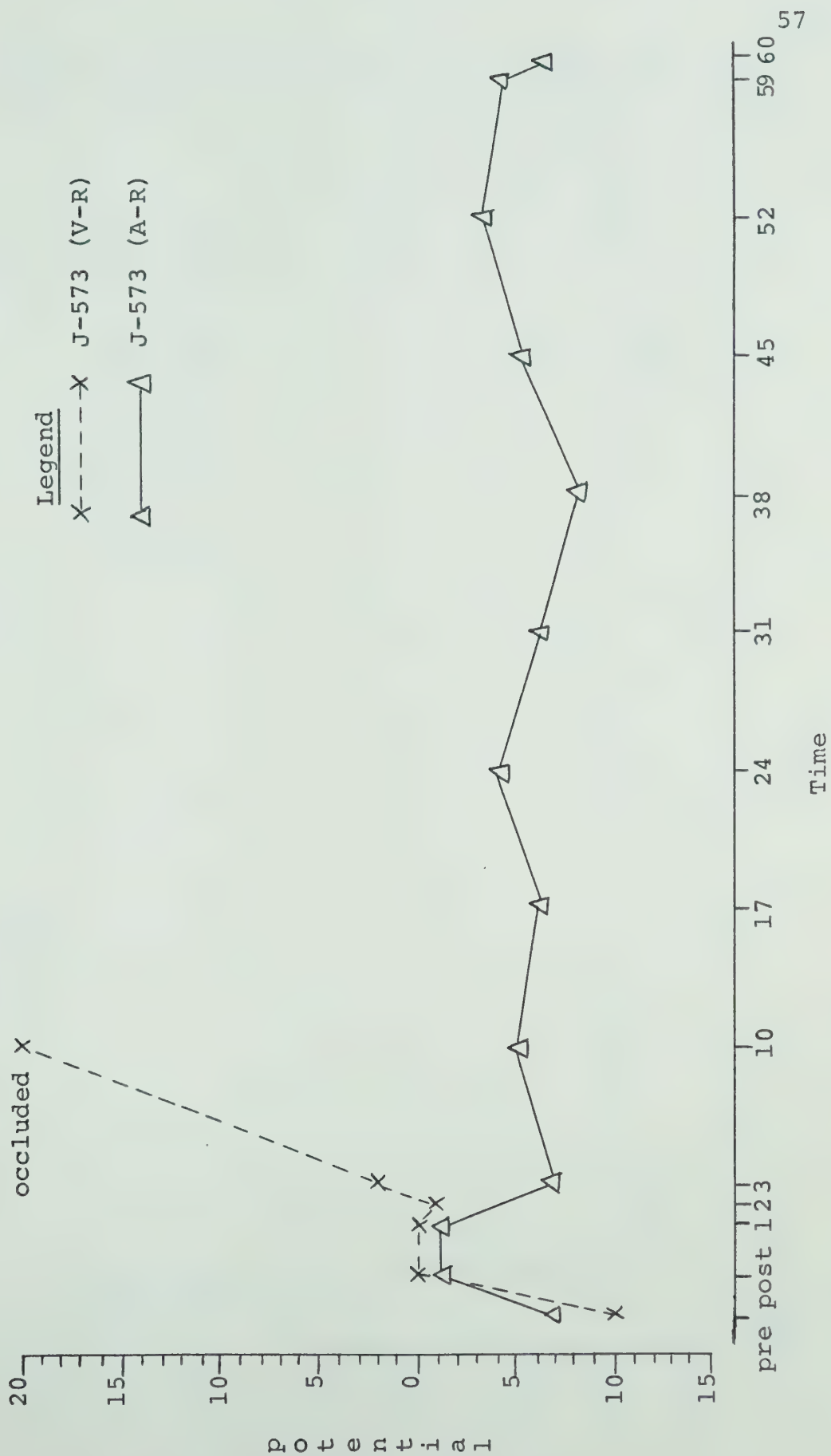
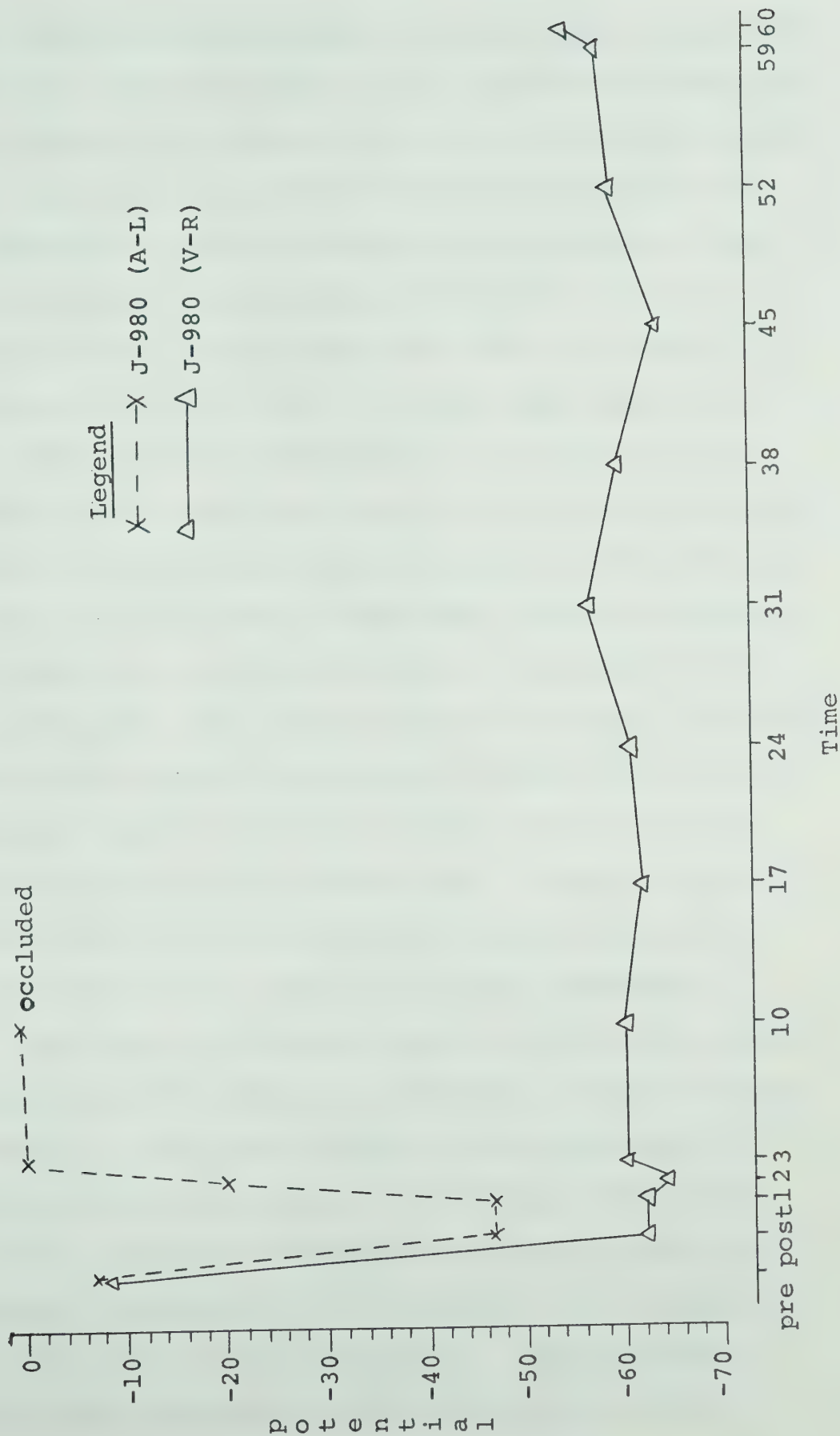


Fig. 5.1.2.2

Induced transmural potentials
in patent and occluded grafts



operative day the flow was found to be minimal. In the case of J-925 (V-R) infection occurred on the first postoperative day and increased in intensity until the twenty-fourth postoperative day when examination showed that the distal end of the anastomosis had been destroyed, most likely by bacterial macrophages. At the time of examining these two anastomosis and on finding them inoperable, the vessel in question was ligated to allow continued experimentation with the other prostheses implanted in the animals. No detrimental effects were noted upon ligation of the vessels, indicating no lack of adequate vasculature. This was most likely due to the presence of redundant vessels or development of colateral vessels in the area of anastomosis.

There was very little change from normal, (for the pH measurements), except in the case of dog J-925 where the blood became slightly acidotic over the interval between the second and tenth postoperative day. Since at this pH the formed elements of the blood would be losing their electronegativity (60), resulting in graft occlusion.

The change in platelet count for this group, except in the case of J-925, was not as dramatic as that of group I. In the case of J-925, where infection had destroyed the anastomosis, there was a drop to 246,000 per cubic millimeter for the immediate postoperative count and again on the third day postoperatively, as compared to a preoperative value of 249,000 per cubic millimeter. This coincides with the initial decrease in pH and the first positive

potential measurement in the eventually occluded graft. There was no dramatic platelet count change for J-980. This can only be explained by the fact that the wire broke some time between the tenth and seventeenth day and occlusion occurred in this interval. No tests were undertaken to determine the changes taking place during this interval.

In this set of experimental implantations the clotting time increased immediately postoperatively but fell to the lower limits of normal on about the third postoperative day, for the case of J-925, and did not return to mid-normal until the twenty-fourth postoperative day. The continued normal clotting times in dog J-980 are a mystery since it would be assumed that as the graft occluded, since there was a rather dramatic drop from patency to occlusion, there would be a lowering of the clotting times in conjunction with the onset of the coagulation system.

The prothrombin times followed a pattern similar to the clotting times with a drop in J-925 and remaining relatively constant and well within normal limits for J-980. This discrepancy could possibly be resolved with further tests but at present must remain unsolved.

Fibrinogen concentrations rose in the case of J-925 to a value of 469 milligrams percent on the first postoperative day but then dropped to a normal value on the third postoperative day. This would seem to be a quite normal reaction since all other surgical procedures pro-

duced a decreased fibrinogen concentration which would indicate initiation of the healing process due to activation of the coagulation process and thus increased conversion of fibrinogens to fibrin. A similar pattern was established in J-980 with normality being reached on the third post-operative day after an initial increase to a maximum of 470 milligrams percent.

5.1.3 Interpolation With Opposition

This group was also designated, as were groups I, IV, and V as a control group. The discussion of the data is identical to that of group I with transmural potentials being in the normal range preoperatively, slightly less negative or zero immediately postoperatively and returning to normal by the second or third postoperative day. All other results; pH, platelets, clotting time, prothrombin time and fibrinogen concentrations can be discussed in a manner similar to that of group I, making the discussion redundant, and thus will not be reviewed here.

The one exception to this is J-573 (V-R) where the graft was found to be occluded on the seventeenth postoperative day. Upon examination the anastomosis proved to have a constriction, due to surgical error, on the proximal end and this was considered to be the cause of the eventual occlusion.

Transmural potential for J-573 in the case of the venous anastomosis on the right side was normal (-10 milli-

volts) preoperatively but only returned to a small negative value (-1 millivolt) on the second postoperative day. There was then a reversal of sign on the third postoperative day (+2 millivolts) with a maximum positive potential of 20 millivolts on the tenth postoperative day.

All other tests, as was previously mentioned, had only predictable changes associated with this one failure.

5.1.4 Graphited Latex

The graphited latex (no battery connected) and the following group (pure latex) were acute experiments lasting, at the longest, 4 hours.

Group IV consisted of latex grafts coated with graphite which occluded between 2 and 4 hours postoperatively. Consequently there are only preoperative and postoperative results to be considered.

The preoperative potentials for group IV ranged from -4 to -18 millivolts, while the postoperative measurements were in the range of 0 to +12 millivolts with the higher, positive measurements clotting faster than the lower potentials. The postoperative potentials near zero in this group might be attributed to the presence of the graphite on the internal side of the prosthesis. This conductive coating could possibly dissipate some accumulated positive charges to the ends of the graft in a manner similar to that of the normal injury current or potential.

All other tests followed a typical pattern with pH

staying constant, platelet count dropping from normal pre-operatively to a lower value postoperatively although not out of the normal range, clotting times dropped to a sub-normal value, as did prothrombin time and fibrinogen concentration, as would be expected in the healing process.

5.1.5 Pure Latex

This group of graft implants reacted in a similar manner to the graphited prostheses but in a more dramatic nature, with the longest period of patency being 2 hours after insertion.

Transmural potentials postoperatively proved to be the most positive in this group, +9 to +20 millivolts, most likely due to the accumulation of positive ions on the inner surface of the prosthesis.

Results for platelets, clotting time, prothrombin time and fibrinogen concentration behaved in the same manner as those in group IV only with a more pronounced change to values well outside normal levels. The values for pH remained constant as would be expected.

The basic premise that there is a repulsion of the formed elements of the blood in a normal vasculature has been discussed in Chapter 2. The results of group II experiments shows that this is a workable thesis in the case of prosthetic materials.

Although there are not enough trials for statistical observations, the data presented here indicates that the

use of latex vascular prostheses is a workable idea if the proper blood interface is achieved.

The data for group II in table II shows there is a relatively large negative potential associated with the type of prosthesis used and although this did not seem to have any detrimental effects, the use of a smaller battery, both electrically and physically, might prove to be more efficient and practical.

In comparing the data of all 5 groups the charged grafts showed the least deviation from normal and were comparable to groups I and III, the living tissue implants. The only incongruities are with the direction of swing. In most cases it was found that platelets clotting time, prothrombin time and fibrinogen concentration decreased while in the case of the charged graft all of these parameters had a slight rise. This phenomena may be explained by the excessively negative transmural potential as shown in figs. 5.1.2.1 and 5.1.2.2 and might be construed as an indication for use of an electrically smaller potential source in order to keep these parameters closer to normal.

5.2 SUMMARY

The change of potential difference from a normally negative value to a value close to zero, or positive, after graft implantation, is the result of normal physiological changes which occur in the surrounding tissues after injury. The major difference between alloplastic materials and living tissue used for reconstructive surgery is the ob-

vious lack of ability of alloplastic materials to present a physiologically acceptable electrical image, as living tissue would. Even if all other parameters such as chemical, physical, nonreactivity, noncarcinogenicity etc. are met, the lack of this electrical image in the case of vascular prostheses has been shown to be a determining factor in the failure rate of vascular prostheses.

5.3 CONCLUSIONS

The inability of a foreign surface to maintain patency in vascular prosthetic applications has been proven in this thesis in groups IV and V where survival of a graphited latex and pure latex graft respectively was 4 hours at the most.

The use of a prosthesis with an electrically conductive inner lining and an associated power source, group II, has been shown to be as effective as natural living tissue for vascular prosthetics.

The living tissue prostheses, transection with end to end anastomosis and interpolation of a segment, had 100% and 75% patency rate respectively.

The charged prosthesis also had a patency rate of 75% being comparable to the interpolation with opposition group thus providing a means of implanting a vascular prosthesis resulting in normal physiological function.

5.4 SUGGESTIONS FOR FURTHER RESEARCH

The major applications of the principle that has

been developed are in the field of surgery, with particular emphasis on:

- (i) Reconstructive peripheral vascular procedures.
- (ii) Local control of diffuse bleeding.
- (iii) Intracardiac insertion of prosthetic valves and artificial hearts.

The first of these has already been demonstrated practical in this thesis. Further work would consist of determining the proper voltage and current to be applied. Along with this could be the study of how small a vessel could be kept patent for a predetermined length of time. These studies could also involve more work on the biochemical side in reference to the exact method of thrombosis prevention and neointimal formation. With this in mind it would now be appropriate to discuss the idea of incorporating an alloplastic material into the body to become living tissue. This possibility could be achieved by first determining if a living intima could be developed on the prosthesis described in this thesis. This might be accomplished by first applying a small positive potential to the graft for a short period of time in order to accumulate a layer of protein on the inner surface of the prosthesis and then applying a larger negative potential to prevent complete thrombosis. In this manner it would be hoped that a living intima could be grown with its own blood supply obtained from the ends of the normal vessel. If this neointima could be developed then it would be a simple matter

of developing a material, to replace the latex, that would dissolve in the body after several weeks or months, leaving only living tissue in the excised segment of vessel. The electrical apparatus for applying positive and negative potentials could be implanted subcutaneously, much like the pacemaker in use today, and removed by a simple skin incision at the appropriate time.

The second area of future research dealing with local control of diffuse bleeding is based on the evidence that a thrombus forms under a positive electrode, this technique could be applied clinically to produce haemostasis in friable tissues such as liver and brain where use of ordinary cautery techniques might prove too destructive. Patients with abnormal bleeding states, such as haemophilia, could possibly benefit from the use of electrodes implanted into wounds or severed vessels and connected to the positive pole of a direct current source.

The third area of future research would be applicable to artificial organs which tend to produce thrombi when implanted. These include artificial heart valves which often occlude due to their presence in the blood stream. These could be made conductive by some method and connected to the negative pole of a battery to prevent thrombus formation. The same procedure could be applied to artificial hearts which are notorious for their blood destroying capabilities. These devices could have a neointima developed on their blood interface, by the method previously discussed, in an attempt to produce a compression surface less injurious to

the elements of the blood, especially red blood cells. In this same area the internal surfaces of extracorporeal heart lung machines could be made conductive and thus rendered anti-thrombotic and possibly less destructive with the ingrowth of a pseudointima.

Other areas of interest might also be developed upon further experimentation in the above areas.

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APPENDIX I

LIST OF SYMBOLS

a	distance above the edge of the cylinder
$\ddot{a}$	acceleration
$\tilde{a}$	elemental vector in the X direction
$\tilde{a}_y$	elemental vector in the Y direction
$\tilde{a}_z$	elemental vector in the direction of axis of cylinder
$\text{\AA}$	Angstrom
AHG	anti haemophilic globulin
A_p	average area of a single dipole
d	thickness of diffuse double layer
dA	differential surface
$d\tilde{P}$	differential dipole element on a differential surface
e	electronic charge
$\tilde{E}$	electric field
E_z	electric field from the cylinder
E_y	electric field along the axis of the cylinder
$\tilde{F}$	force
K	Boltzman's constant
L	length of the cylinder
m	mass
M_i	molar concentration: $i = 1, 2, 3$
n	number of ions per cubic centimeter
$\tilde{P}$	dipole density function

$\tilde{P}$	dipole moment
P_x	component of dipole moment in X direction
P_z	component of dipole moment in Z direction
PTA	plasma thromboplastin antecedent
PTC	plasma thromboplastin component
Q	a charge on the axis of symmetry
r	radius of an elemental area
$\tilde{R}$	distance of dipole from axis of cylinder
T	absolute temperature
V	potential of a point dipole
X	radius of the cylinder
Z	valency of the ions
α	angle between R and Z
α_L	$\cot^{-1} (-a/X)$
α_S	$\cot^{-1} ((L-a)/X)$
ϵ	dielectric constant of the medium
ϵ_0	dielectric constant of air
δ	thickness of compact part of diffuse layer
ψ	potential of the surface
ψ_c	critical potential at the surface
ψ_0	normal potential at the surface
ψ_δ	potential between Stern and Gouy layer
ξ	Zeta potential

APPENDIX II

II-1 BIOPHYSICAL ASPECTS OF ELECTROSTATIC REPULSION IN THE VASCULAR SYSTEM

In recent years there has been a tremendous amount of research carried out on biological membranes, one aspect of which is the biophysical make-up of the surface. Numerous structural models of biological membrane systems have been proposed to explain a wide variety of intra-and extra-cellular functions. Both analog and molecular models have been brought forward to explain phenomena dealing with motility, excitability, transport, permeability, adsorption, adhesion and other biochemical processes. Models of the molecular structure of biological membranes are necessarily based upon incomplete data at a molecular level and real structures, complex as they are, are often considered by making specific assumptions as to which variables may be neglected.

Within the past decade, by far the greater number of experimental membrane studies have centered around artificial perm-selective membranes (21,97). These membranes vary from simple chemical ion-exchange columns (97) and specifically prepared synthetic films (32,68,71,81,86,116), to specific artificially prepared membranes of biological lipid origin (78). The basic principles arising out of these studies involve the elucidation of structure necessary

to perform a given chemical selective function. Thus several studies have yielded information dealing with chemical selectivity. Investigations have included a variety of cellulose membranes along with their electron micrographs (86), liquid anionic and cationic exchange membranes along with their electron micrographs and polystyrene membranes (21).

Analysis of these varies from simple temperature dependence through electrical measurement to application of the double layer theory (70).

More theoretical emphasis has been given to artificial membranes by considering the adsorption isotherms associated with a large number of surface phenomena (59). Often, also, biologically unrelated theoretical studies can be applied to yield useful information. Numerous examples (54) may be found in the literature where desorption and adsorption of thin organic films of diverse macromolecular structure on metal surfaces have been investigated. Studies on coacervation (88) may also pertain.

The electrostatics of adsorption has been sparsely treated focusing only upon specific geometrics and special systems (57). A review of the additivity of intermolecular forces at interfaces is given by Fowkes (42). The analysis of these systems classically involves double layer theory and Van der Waal's interactions.

Recent interest in the role of metal ions binding to artificial membraneous structures and lipid constituents has given new emphasis to complex formation in membrane

structure and function (23). Binding and complexing properties of calcium, magnesium and heavier metals with membraneous materials and lipid fractions of brain tissue have only recently been investigated (47).

Functional analogs of transport phenomena for biological membranes have been well characterized in the literature for some time (122). Ion transport in particular has received much attention, usually without reference to structural features of a membrane model (58). Thus, diffusion equations, conservation of charge, and net flux equations are the basis of such analyses. Recently, attention has been given to problems of permeability and excitability in terms of analog models based on membranes of limited structural identity (8). Of special note is the work of Rudin and Mueller (75) with artificial membranes which parallel known biological structures that have been reconstituted from lipid materials.

More classical approaches using experimental data on the erythrocyte membrane have suggested models accounting for mechanical properties. These models range from springs and dashpots to mathematical models of elasticity and strain (92).

Evidence which has been accumulated from a diversity of sources for a limited biological membrane composed of a protein-coated bimolecular layer of lipid and phospholipid, has created a framework for membrane structure on which most investigators agree. In addition, there is a general agreement that the membrane is roughly 100 to 1000 Angstroms

thick. There is also general qualitative agreement that the bilayer contains cholesterol, galactosides and phospholipids of which sphingomyelin, phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, diphosphoglyceronositide are the major components. The hydrocarbon chains are directed toward the interior of the bilayer and the polar groups are exposed on both sides in contact with the protein.

With this very simple picture of the membrane structure in mind, it is necessary to consider in more detail the evidence for certain biophysical aspects of the model in use. These details to be considered are:

- (i) Dimensions of the bilayer.
- (ii) Molecular constituents and packing of the bilayer.
- (iii) Orientation of the lipid molecules in the lipid bilayer.

II-2 Dimensions of The Bilayer

Within the past few years much interest has been shown in "black films" formed by soap and lipid materials. Studies by Rudin and Mueller (76), Overbeek (84), Thompson (55) and Haydon (48) have shown that these artificial bilayer films are comparable in dimension and electrical properties to the range of dimensions and properties of their biological counterparts. Although a similar variation in the width of the bilayer is reported from one investigator to another, as in the biological case, well sub-

stantiated evidence has been gathered on the packing of the molecules in these artificial bilayers. Thus, the hydrocarbon chains are considered to be perpendicular to the plane of the membrane, while the polar groups have freedom of orientation with external fields depending on the molecular packing. The density has been firmly fixed at between 20-30 Angstroms² per chain resulting in an average packing area of 25 Angstroms² per molecule (48,55,76,84).

II-3 Molecular Constituents and Packing of The Bilayer

A number of studies (96,99) have identified enzyme constituents associated with the biosynthesis of bilayer constituents. Rothfield (96) has studied the lipid composition of the cell wall as it relates to enzyme components for the biosynthesis of bacterial lipo-polysaccharides in the cell wall. Investigations of the lysosomal membrane indicate that this bilayer has the characteristics of a charged membrane (99).

Specific analyses of lipid components of membraneous structures are most often performed on erythrocytes and macrophage membranes (29,83). Labeling techniques are now common and yield data of the percent lipid component in the bilayer (83). It has been shown that the cholesterol-phospholipid ratios of lipids of butanol-treated erythrocytes is significant in relation to permeability (115). Investigations of micelle formation of lipids and related substances cannot be overlooked in understanding the stability and the formation of the bilayer relative to its chemical

composition. Studies in polar and non-polar solvents with micelles of Lecithin indicate dimensions of interesting head groups and conversions of monomolecular leaflets to bimolecular types (39). The relationship of the dielectric constant of these micelle groups to permeability is also analysed. The use of anionic, cationic and non-ionic detergents in micelle formation, yields data on the limiting size of the aggregates relative to their charge properties (25). By examination of spontaneous binding of protein from mitochondria with micellar phospholipids a possible mechanism of genesis of bilayers in the cell (94) can be inferred.

The packing of chemical constituents in the bilayer of biological membranes has been sparsely considered. Research concerned with molecular sieving by cell membranes of *Bacillus megaterium* indicates a hexagonal arrangement in the structure of lipid bilayers (111).

II-4 Orientation of Lipid Molecules in The Bilayer

Early surface chemical studies on the monolayer of lipid substances at air-oil and water-oil interfaces elucidate the basic structural orientation of molecules in liquid and condensed phases. Potential measurements, although crude and often contradictory in those early investigations, sustained a picture of lipid molecules in which the hydrocarbon tails are perpendicular to the plane of the water molecules in a condensed phase, and slightly tilted in the liquid phase (111). Many of these force-area and potential-

area curve measurements are still useful today. It has been shown from these early studies that the larger the area per molecule the flatter it lies in the plane. Changes in the potential during this tilting process indicate a change in the orientation of the polar end group which has been theoretically substantiated by analysis of the orientation angle of the dipole moment. A similar effect was obtained by changing the concentration of electrolyte in the aqueous solution. On comparing bilayer and monolayer molecular densities the usefulness of this data becomes apparent. Thus with molecules packed on 25 Angstroms^2 per carbon chain bordering on a transition between liquid and condensed phases, the polar groups and/or hydrocarbon chains, on strictly geometric grounds, have sufficient room to tilt in aligning with an external field.

In turning to more recent monolayer studies with both ionized and dipole polar end groups, depth of immersion of the head group in the aqueous phase has been established (66). However, interest in the orientation of the polar group has not appeared. The influence of charged polar groups of monolayers on protein molecules has also been investigated (62). In general, references ignore the influence of the dipole end groups of lipid molecules as being an insignificant and negligible factor in electrostatic calculations (42,49). Also, research on transmembrane potentials in living biological membranes attempts to explain the data solely in terms of ionic concentrations within the cellular vacuole (89,118). Equally incorrect

are molecular models of the membrane which treat theoretically the protein layer as the only electrostatic component (50). Similarly the influence of external electric fields on the bilayer structure has not been experimentally established.

A number of structural models, which in some ways are related to the bilayer structure have been proposed to explain special functions. Daniel (28) analyzes the high intensity electric field properties of linear chains of dipoles of solid secondary alcohol from a statistical point of view. From this analysis, dielectric saturation in chains of hydrogen bonds is used as a model for field controlled ion trapping in a mechanism of conduction in nerve membranes.

A model of dipole orientation and polarization is presented in a theory of dielectric crystalline dispersion by Yamafugi (128). Although this theory is far removed from molecular bilayer structure, it is of interest to note that even in solid macromolecular crystalites, reorientation of dipoles plays a major role in field effects.

One of the most noteworthy and comprehensive representations of the biological membrane has been set forth by Hechter (50). His theoretical considerations, at the molecular level, of the water molecules in and around the bilayer, as well as his analysis of configurations and clathrate structures of water coupled with hexagonal and pentahedral configurations in the packing of the protein monolayer is marshalled with much theoretical and experi-

mental evidence. The influence of the bilayer itself as it interacts at the molecular level with this hexagonal sub-unit model of water and protein is as yet to be determined.

A model taking into account orientation effects of the dipoles in the polar end groups of the bilayer is proposed by Goldman (44). He presents a molecular structural basis for the excitation properties of axons. His structural model consists of a double layer of lipid and phospholipids which change their orientation and combining properties under the influence of an electric field. The driving forces are assumed to be electrostatic forces and concentration gradients. The electrostatic is treated from the point of view of statistical mechanics with structural models of the phospholipids. Justification of the dipole orientation is given in terms of the atomic configuration of the polar end groups of the complex phospholipids.

Any structural model used to explain biological membrane phenomena must satisfy, in the first instance, the molecular configuration of the bilayer. If the framework of all biological membranes is a bimolecular lipid leaflet 20-100 Angstroms across, then a "molecular model" is defined which represents in a "chemical" sense the real lipid and phospholipid molecules found in the actual configuration of the bilayer. For this model to be valid it must also include water molecules and electrolytes, which are always present, as well as macromolecules of protein adjacent to both sides of the bilayer (50). Since at this time, these entities are structurally uncertain at the molecular level,

it is not possible to identify these variables in any great detail.

From the "molecular model" it is necessary to abstract electrostatic properties of first order effect that will constitute an "electrostatic" or "physical" model. Thus initial analyses will neglect the hydrocarbon chains in the bilayer which give rise to London-Van der Waal's dispersion forces which vary as $1/r^7$. Charge and dipole effect which vary as $1/r^2$ and $1/r^3$ must be taken into account. Therefore, the "physical model" of the bilayer will consist of a double layer of discrete dipoles, whose actual dimensions and dipole moments are taken from the real lipid molecules of the "molecular model". Since the bilayer often contains a net negative charge, due to the partitioning of the electrolyte or ionization of polar groups, a fixed charge density may also be superimposed.

It has been well established (77), that the stability of the bilayer resides in the attractive forces (London-Van der Waal's dispersion forces) of the hydrocarbon chain as opposed to the repulsive forces of the two extreme layers of dipoles. Experimental data leading to these facts have been gained from studies of lipid micelles and early investigations of Debye (31). Thus the hydrocarbon chains "screen out" the effect of the charge centres beneath the bilayer so that only one layer of dipoles need be considered.

Using this background it is possible to calculate the electric field intensity in almost any configuration of

dipoles. The electric field intensity for the simple case of axial electric field in an open cylinder will be derived here. The cylinder was chosen, over planar surfaces, since this work is to apply mainly to vessels and electrostatic repulsion of the formed elements of the blood to prevent thrombosis.

The general equations for the electric field intensity and potential energy for a point dipole function (for a single dipole) will be derived such that the equations are immediately integrable over a cylindrical surface. In the general equations, the point dipole function is a moment function, while in the cylindrical equations, the dipole function becomes a moment density function. Thus:

$$d\tilde{P} = \frac{|\tilde{P}|}{\tilde{A}_p} dA = \tilde{p} dA \quad 1$$

where:

$\tilde{P}$ = dipole moment

$\tilde{A}_p$ = average area for a single dipole

$d\tilde{P}$ = differential dipole element on
a differential surface dA

$\tilde{p}$ = dipole density function

The dipole density function will have radial and Z-directional components. The tangential component will not be considered. Thus this derivation only applies to the axis of symmetry of the cylinder.

The potential of a point dipole is given by:

$$V = \frac{P \cdot R / |R|^3}{4\pi\epsilon_0} \quad 2$$

From fig. II-4.1 the following vector equations are obtained:

$$\tilde{\mathbf{P}} = \tilde{P}_X \hat{\mathbf{a}}_X + \tilde{P}_Z \hat{\mathbf{a}}_Z = |\tilde{\mathbf{P}}| (\sin\theta \hat{\mathbf{a}}_X + \cos\theta \hat{\mathbf{a}}_Z) \quad 3$$

$$\tilde{\mathbf{R}} = X \hat{\mathbf{a}}_X + X \cot\alpha \hat{\mathbf{a}}_Z \quad 4$$

$$\tilde{\mathbf{P}} \cdot \tilde{\mathbf{R}} = |\tilde{\mathbf{P}}| X \sin\theta + \tilde{P}_Z X \cos\theta \cot\alpha \quad 5$$

$$|\tilde{\mathbf{R}}| = (X^2 + X^2 \cot^2\alpha)^{1/2} = X \operatorname{cosec}\alpha. \quad 6$$

Substituting eqs. 5 & 6 into eq. 2 and simplifying, yields:

$$V = \frac{|\tilde{\mathbf{P}}|}{4\pi\epsilon_0 X^2} (\sin^2\alpha \cos(\alpha-\theta)). \quad 7$$

To determine the field, $\mathbf{E} = \nabla V$, where:

$$\nabla = \frac{\partial}{\partial X} \hat{\mathbf{a}}_X + \frac{1}{X} \frac{\partial}{\partial Y} \hat{\mathbf{a}}_Y + \frac{\partial}{\partial Z} \hat{\mathbf{a}}_Z. \quad 8$$

Since E_Y is perpendicular to X and Z (right hand rule) it is given by:

$$E_Y = -\frac{1}{X} \frac{\partial V}{\partial Y} = 0. \quad 9$$

Utilizing eqs. 7 & 8, the field in the X direction is given by:

$$E_X = \frac{|\tilde{\mathbf{P}}|}{2\pi\epsilon_0 X^3} (\sin^2\alpha \cos(\alpha-\theta)). \quad 10$$

To evaluate E_Z , from fig. II-4.1, we see that:

$$Z = X \cot\alpha \quad 11$$

Z: Z is the axis of the cylinder

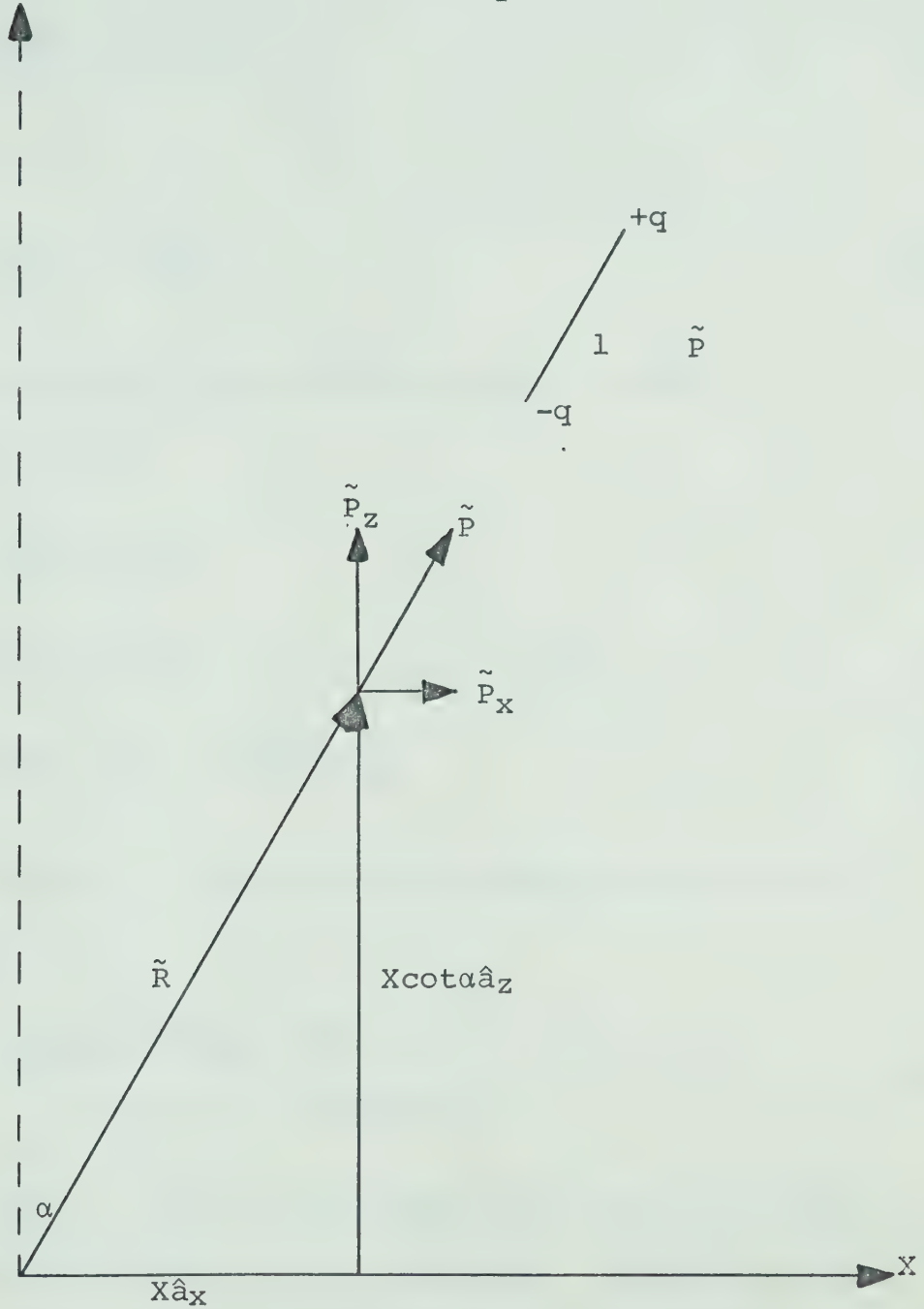


Fig. II-4.1

Dipole density components
on surface of cylinder

and

$$\frac{\partial X}{\partial \alpha} = -X \operatorname{cosec}^2 \alpha \quad 12$$

or

$$\frac{\partial \alpha}{\partial Z} = - \frac{\sin^2 \alpha}{X}. \quad 13$$

Applying the chain rule to the gradient in the Z-direction gives:

$$\frac{\partial V}{\partial Z} = \frac{\partial V}{\partial \alpha} \frac{\partial \alpha}{\partial Z}. \quad 14$$

Now incorporating eq. 14 into eq. 8 yields:

$$E_Z = - \frac{\partial V}{\partial Z} = - \frac{\sin^2 \alpha}{X} \frac{\partial V}{\partial \alpha}. \quad 15$$

Substituting eq. 7 into eq. 15 and simplifying gives the result:

$$E_Z = \frac{|\tilde{P}|}{4\pi\epsilon_0} \frac{\sin^2 \alpha}{\tilde{X}^3} (3\sin\theta \sin^2 \alpha \cos\alpha + \dots \dots + \cos\theta \sin\alpha (3\cos^2 \alpha - 1)). \quad 16$$

Eqs. 9, 10, 16, represent the field at a point due to a single dipole on the cylinder. Summing around cylinder will now give a cylindrical dipole configuration.

An element of field $d\tilde{E}$ may now be defined, whose origin is an elemental dipole $d\tilde{P}$ as:

$$d\tilde{E} = \frac{\tilde{E}}{\tilde{r}^3} d\tilde{P} \quad 17$$

where E is the total field of eqs. 9, 10, 16.

Thus $d\tilde{E}$ will be the element of field at point "P" a distance and direction $\tilde{R}$ from the elemental dipole (see fig. II-4.2). In order to determine the total field at point $\tilde{P}$ from all the elemental dipoles on the cylinder it is necessary to integrate over the surface thus:

$$\tilde{E}_{\text{total}} = \int_s d\tilde{E} = \int_s \frac{E}{\tilde{R}^2} d\tilde{P}. \quad 18$$

Recalling that:

$$d\tilde{P} = \tilde{p}dA$$

the differential area element of a cylinder is given by:

$$dA = r \, dy \, dz. \quad 19$$

In order to establish the limits of integration the following substitution is made:

$$dz = \frac{\partial z}{\partial \alpha} d\alpha + \frac{\partial z}{\partial r} dr$$

since $dr = 0$,

$$dz = \frac{\partial z}{\partial \alpha} d\alpha. \quad 20$$

But from eq. 12:

$$\frac{\partial z}{\partial \alpha} = - \frac{X}{\sin^2 \alpha}.$$

Thus:

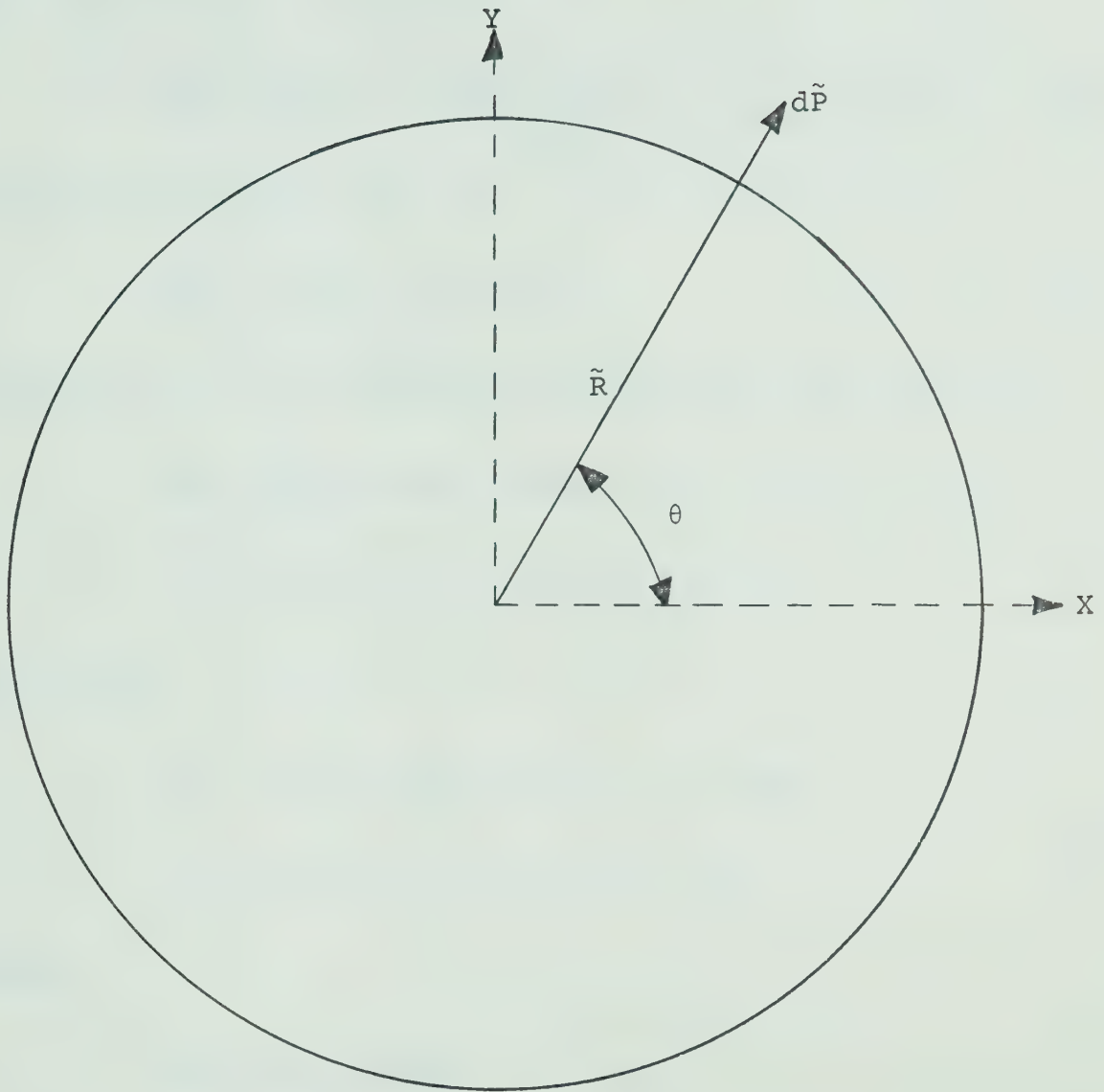


Fig. II-4.2
Cylindrical configuration of a dipole

$$dz = - \frac{X}{\sin^2 \alpha} d\alpha. \quad 21$$

Considering first the radial component, substitute eq. 10 into eq. 17 yielding:

$$dE_x = \frac{\tilde{E}}{\tilde{p}} d\tilde{p} = - \frac{\sin^2 \alpha \cos(\alpha - \theta)}{2\pi\epsilon_0 X^3} \tilde{p} \times dy dz. \quad 22$$

Thus from eqs. 18 & 22:

$$E_x = \int_{y=0}^{\pi} dE_x + \int_{y=0}^{-\pi} dE_x = 0 \quad 23$$

Considering the differential Z-field from eqs. 16 & 17:

$$dE_z = \frac{\tilde{p}}{4\pi\epsilon_0 X} (3\sin\theta \sin^2 \alpha + \dots \dots + \cos\theta \sin\alpha (3\cos^2 \alpha - 1)) dy d\alpha. \quad 24$$

Therefore:

$$E_z = 2\pi\tilde{p} \int_{\alpha_S}^{\alpha_L} \frac{1}{4\pi\epsilon_0 X} (3\sin\theta \sin^2 \alpha \cos\alpha + \dots \dots + \cos\theta \sin\alpha (3\cos^2 \alpha - 1)) d\alpha. \quad 25$$

where:

$$\alpha_S = \cot^{-1}((L-a)/X) = \alpha_{\min}.$$

$$\alpha_L = \cot^{-1}(-a/X) = \alpha_{\max}.$$

L = length of cylinder

a = distance above the edge of the cylinder

X = radius of the cylinder

Performing the integration with respect to α gives the E field on the axis of symmetry of a cylindrical config-

uration of dipoles at a point which is at a distance "a" above the edge of the cylinder of length L.

Thus:

$$E_Z = \frac{|\tilde{p}|}{2\epsilon_0 X} (\sin^2 \alpha_L \cos(\alpha_L - \theta) - \sin^2 \alpha_S \cos(\alpha_S - \theta)) \quad 26$$

Hence we see for the special case mentioned above:

$$E_Y = E_X = 0$$

$$E_Z = \frac{|\tilde{p}|}{2\pi\epsilon_0 X} (\sin^2 \alpha_L \cos(\alpha_L - \theta) - \sin^2 \alpha_S \cos(\alpha_S - \theta))$$

Now by a simple analysis of the dynamic properties of this system utilizing Newton's second law and charge dynamics and neglecting drag for this simple case.

$$\tilde{F} = m\tilde{a} \text{ and } \tilde{F} = Q\tilde{E} \quad 27$$

yields:

$$\tilde{a} = \frac{Q}{m} E_Z \quad 28$$

where:

$\tilde{a}$ = acceleration

Q = a charge on the axis of symmetry

E_Z = field from the cylinder

It is now of interest to consider the motion of a charged particle at one end of the cylinder. If the dipoles are aligned so that they are perpendicular ($\theta=90^\circ$) to the cylinder, the charged particle will accelerate towards the

axis of the cylinder where it will decelerate and eventually come to rest. In this way if the surface of the vessel is negatively charged, as it is thought to be, with respect to the adventitia or to the blood, the negatively charged formed elements of the blood will migrate to the centre of the vessel resulting in no adhesion and hence no thrombus formation.

From this review of the data and theory, known to date, it can be seen that the biophysical make-up of the cell wall can, and probably does, play an important role in haemostasis. This same theory may be extrapolated to other membrane systems in a living species, for example the same derivation as presented above may very well be applied to the conduction of nerve impulses along nerve fibers (44).

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